Breeding system evolution in relation to adult sex ratios

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Summary

The study of breeding system evolution has been prolific given the broad subjects that it embraces, from the social environment and sexual selection to courtship behaviour and parental care. The general objective of this dissertation was to test empirically ideas shown in recent models of breeding system evolution. Using plovers *Charadrius* spp. as model organisms, which show remarkable variation in breeding systems, I investigate how adult sex ratio (ASR) and operational sex ratio (OSR) are linked together and relate to two major components of breeding systems: courtship behaviour and parental care. Experimental studies have led to equate ASR and OSR, but they are not necessarily the same and the way they correlate in nature is unknown. Here I show that in a wild polygamous plover population these ratios seem to be uncorrelated (Chapter 2). Differences in the social environment and the degree of mating competition between monogamous and polygamous populations could also be reflected in their courtship behaviour; higher courtship rates in a polygamous plover than in a monogamous closely-related plover support this prediction (Chapter 3). OSR may be more variable throughout time than ASR, and therefore be less reliable as a cue to the social environment. In support of this prediction ASR was better than OSR at predicting the duration of female brood care in a polygamous plover where females have variable care (Chapter 4). Sex-specific timing of breeding could differ according to the social environment and the level of mating competition in a population, I compared six populations of five closely-related plover species with varying breeding systems (Chapter 5); only one polygamous population showed sex-specific differences in arrival date and the length of time spent in the breeding grounds. Finally, I discuss the contribution of these results to the understanding of breeding system evolution and suggest potential future lines of research.

1 Introduction

Breeding systems

Breeding systems comprise any behavioural strategy to obtain mates, reproduce and care for progeny in any sexual animal (Reynolds 1996). It describes the mating strategies: form of courtship and competition, number of mates, duration of pair bonds and the post-mating behaviours like the form and duration of parental care (Reynolds 1996). Although originally referred to as mating systems in previous literature (e.g. Darwin 1871; Emlen and Oring 1977) the difference between these two terms, as Reynolds (1996) suggests, relies in the reproductive stage one wants to address: the mating strategies ('mating system') or both the mating strategies and the post-mating behaviours ('breeding system').

In most species, male's and female's competitiveness for mates (i.e. sex-roles) are different, usually being males the most competitive sex (Parker and Pizzari 2015) and these differences have typically been attributed to the size of the gametes (Lehtonen et al. 2016) and the level of parental investment ("any behaviour that increases offspring survival at the cost of parent's survival or future reproduction", Trivers 1972). Early theory suggests that these differences in parental investment create a mating differential between the sexes, giving rise to different costs and benefits for males and females that shape the different mating strategies and different sex-roles found in nature (Andersson 1994; Alcock 2005). These theoretical arguments are built upon Bateman's (1948) study in which he showed that males' fitness depends on the number of mates it has, although females' fitness is unrelated to number of mates (but see next section) and can increase with e.g. mechanisms that discriminate between high/low quality mates. Given the reproductive differences between males and females, the sex with the highest parental investment will be a limiting resource for which the other sex will compete for (Andersson 1994). In the non-limiting sex (usually males) sexual selection will be stronger and therefore it will compete for mates and have more elaborated behaviours or ornaments to attract mates. The limiting sex (usually females, species from most taxa of arthropods, fish, reptiles, birds and mammals) will carry out most of the care of offspring and will usually be the choosy one.

Several types of breeding systems exist in nature, however the most general classification is: monogamy and polygamy (for a thorough description of all types of mating systems see: Shuster and Wade 2003). Monogamous populations generally breed with one mate and may

divorce or maintain the pair-bond in subsequent breeding seasons and will generally have biparental care (e.g. 90% of birds), however only 20 to 25% of bird species are both socially and genetically monogamous (Gowaty 1996). On the other hand polygamous populations can have more than one mate in one breeding season and have uniparental care. Although this classification refers to the general patterns observed in a population, it is worth mentioning that cases of polygamy or monogamy may occur in monogamous or polygamous populations respectively. Traditional theory suggests that the difference between monogamous and polygamous populations will depend on the mate-monopolization capacity and variation in the spatial and temporal distribution of mates and/or other resources (Emlen and Oring 1977). Moreover, there is a strong difference in the pressure of sexual selection between monogamy and polygamy suggesting it is stronger under polygamy as suggested by the dimorphism in secondary sexual traits that increases with polygamy, and is subtle under monogamy (Møller and Pomiankowski 1993; Andersson 1994).

Sex ratios, breeding systems and new theoretical models

Bateman's (1948) study influenced modern sexual selection theory and supported Darwin's (1871) view of 'eager males' and 'coy females'. However, criticism to sexual selection theory and of its stereotypical view of sex-roles have arisen (Roughgarden et al. 2006; Gowaty et al. 2012) and evidence of post-ejaculatory selection (e.g. cryptic female choice, sperm competition, Eberhard 1996) and empirical studies supporting the fact that females do benefit from multiple matings (Jennions and Petrie 2000; Simmons 2005; Pizzari and Bonduriansky 2010) have changed the understanding of Bateman's study and a change on the view of the stereotypical passive role of females.

Sexual selection theory predicts that it is the operational sex ratio (OSR, the proportion of sexually active males in the mating pool, Emlen and Oring 1977) the principal determinant of the strength in which sexual selection acts over a population (Emlen and Oring 1977; Clutton-Brock and Parker 1992b; Kvarnemo and Ahnesjö 1996; Reynolds 1996); predicting that sexual selection acts with different strength in monogamy versus polygamy due to a difference in the OSR (Emlen and Oring 1977) and due to anisogamy (Trivers 1972). However, studies criticizing this rigid view have hinted that OSR may not be as consistent as thought (Shuster 2009) and may not generally predict the strength of sexual selection (Klug et al. 2010).

Mayr (1939) first hinted into the relation of biased sex ratios and non-monogamy, and studies relating OSR to breeding systems and sexual selection have since flourished. The OSR will depend on the adult sex ratio (ASR, proportion of males in the adult population) and the rate of reproduction of each sex, being therefore a dynamic property of populations since individuals

may shift between sexually active and inactive periods (Clutton-Brock and Parker 1992b). The rate of reproduction will depend on the sex differences in parental care and mate competition. In monogamous breeding systems the relative rates of reproduction of both sexes are similar given biparental care and low mate competition or equally strong mate competition between both sexes and therefore OSR may not be strongly biased. However, in polygamous species there is one sex whose rate of reproduction is higher given sex differences in reproductive rates and uniparental care, OSR will most likely be strongly biased.

Traditional theory has assumed that OSR and anisogamy predict the intensity of mate competition and sexual selection, determining two things: which sex competes more and which sex provides parental care (Emlen and Oring 1977; Clutton-Brock and Parker 1992b; Kvarnemo and Ahnesjö 1996). Recent theoretical models propose that it is not only OSR and anisogamy which determine competition, care and sexual selection, but also relatedness to offspring, variance in mating success (Queller 1997), and ASR (Jennions and Kokko 2010). Moreover, there are influential and intricate feedbacks between mate competition, parental care and OSR that may have been oversimplified in traditional theory (Székely et al. 2000; Székely et al. 2014) and these intricate feedbacks will also impact ASR. For example in mammalian systems male-biased OSR creates stronger male-male competition which in turn favours female-only care, stronger male-male competition also reduces males survival probability and creates a female-biased ASR.

Recent theoretical models and reviews have tried to acknowledge and stress the importance of ASR in breeding system and sex-role evolution (Donald 2007; Kokko and Jennions 2008; Jennions and Kokko 2010; Székely et al. 2014) as mate competition and parental investment will also be affected by ASR given that mate availability will depend on the total numbers of males and females in the population (Jennions and Kokko 2010). Recent empirical evidence has also diminished the importance of anisogamy over sex differences in parental investment (Liker et al. 2015). Moreover, recent models have found that the way ASR may affect parental investment will depend on how the bias in ASR was originated (Fromhage and Jennions 2016), being potentially more complicated than previously thought. Both ASR and OSR are fundamental demographic properties of a population that encompass an essential part of the social environment in which individuals develop and reproduce.

Recent studies have pointed to the fact that in past literature OSR and ASR tended to be confused and undistinguished (Kokko and Jennions 2008) and have pointed to the relevance of not only OSR but ASR in breeding system evolution (Kokko and Jennions 2008; Székely et al. 2014). Although the relation between OSR and ASR is theoretically straightforward as ASR is a precursor of OSR, they have seldom been studied together, few empirical studies

acknowledge both (Whitfield 1990; Balshine-Earn 1996) and recent models suggest that their correlation may be context-dependent (Fromhage and Jennions 2016).

ASR has its origins in the variation that exists in sex ratios in other life stages (at conception, birth or at independence). Additionally, sex differential mortalities, maturation times, dispersal and migration patterns will all impact on ASR (Bessa-Gomes et al. 2004; Veran and Beissinger 2009; Székely et al. 2014) deviating it from a one to one ratio (see figure 1). Any behaviour that may impact mortalities unequally between sexes will be important in sculpting biases observed in ASR. For instance, OSR which is tightly linked with male-male competition can also be linked with higher male mortalities due to the higher sexual competition confronted by males and therefore a male-biased OSR may be linked with higher male mortality and a higher female-biased ASR (e.g. most mammal species).

ASR has numerous ecological and evolutionary implications (Kokko and Jennions 2008; Veran and Beissinger 2009; Székely et al. 2014), it may impact population growth (Bessa-Gomes et al. 2004; Donald 2007; Veran and Beissinger 2009), mate choice, pair-bonds, mating systems, parental care and paternity (McNamara et al. 2000; Székely et al. 2000; Kokko and Jennions 2008; Liker et al. 2014). For example, unbalanced ASRs are associated with: an increase in male mate-guarding behaviour in the white-fronted chats (Major 1992), higher rates of divorce in invertebrate endoparasites, lobsters, fish, mice and birds (DeLong 1978; Balshine-Earn and Earn 1998; Debuse et al. 1999; Beltran et al. 2009; Karlsson et al. 2010; Liker et al. 2014), and reversed sex roles in shorebirds (males providing most parental care, Liker et al. 2013).

The study of breeding systems is important given its implications in population ecology, sexual selection and conservation. Breeding systems have been widely studied given its broad subject coverage from sexual selection and mating behaviour to parental care. However, although numerous empirical and theoretical studies about breeding system evolution exist, sometimes these contradict each other (e.g. Fromhage and Jennions 2016) or generate controversy (e.g. Ah-King 2013; Lehtonen et al. 2016), proving that our understanding of breeding system evolution is still limited. Empirical studies that provide evidence to support/reject theoretical models of breeding system evolution are needed as they will help clarify which conditions/assumptions of theoretical models are important or realistic, and which predictions are met.

Plovers as a model species

Typically shorebirds have been used to study sexual selection, breeding systems and sex-roles ever since Darwin given the broad behavioural and ecological diversity they present in mating and parental strategies (Szekely and Reynolds 1995; Thomas et al. 2007). In this dissertation I used plover species from the genus *Charadrius,* within the Charadriiformes (shorebirds, gulls, alcids and allies) the genus *Charadrius* consists of 30 species of small plovers that breed on all continents (Dos Remedios et al. 2015). I focus primarily on two species: snowy plover (*Charadrius nivosus*) and Kentish plover (*Charadrius alexandrinus*); and include three more species in one chapter: Kittlitz's plover (*Charadrius pecuarius*), Madagascan plover (*Charadrius thoracicus*), and white-fronted plover (*Charadrius marginatus*). All these plover species provide a good model system to study breeding system evolution for three reasons. First, all species chosen are closely-related (Dos Remedios et al. 2015) and exhibit similar life-histories and ecology: small ground-nesting insectivorous birds associated with large bodies of water and saltmarshes. Second, within the populations of these five species there is ample breeding system variation from monogamous with biparental care to polygamous with male-only care. Third, high quality breeding data is available for populations of all species as they have been studied between four to seven years using the same standard methodologies.

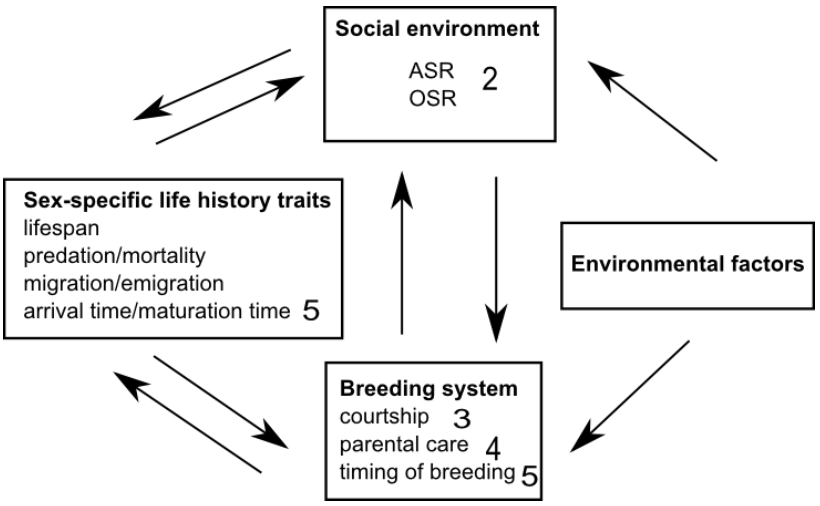

Figure 1. Conceptual framework of breeding systems in relation to social environment, environmental factors and life history traits showing the potential feedbacks between them. The numbers refer to the chapters of my dissertation showing the subjects covered in each chapter.

Dissertation outline

The main objective of this dissertation is to provide empirical evidence to the new theoretical framework that is beginning to develop around breeding system evolution by showing how ASR, OSR and breeding systems relate using several populations of closely related plovers that present variation in ASR and breeding systems.

The chapters presented in this dissertation follow a chronological logic in terms of reproduction (figure 1). First in **Chapter 2**, I describe the social environment in which a polygamous snowy plover population breeds using detailed reproductive data to produce high quality estimates of ASR and OSR to explore their temporality and their correlation. Following the differences in social environment and mate competition that presumably exist between monogamous and polygamous populations, in **Chapter 3** I compare two populations of two closely related plover species (Kentish plover and snowy plover) with contrasting breeding systems (monogamous versus polygamous) and compare their courtship rates. The social environment may also impact the parental care that follows a successful reproduction, so in **Chapter 4,** using the snowy plover population I attempt to see whether ASR or OSR better predict female's duration of care in a polygamous plover where females may abandon broods to re-mate. The social environment may well be cause and consequence of mating patterns and sex-differential breeding behaviours, in **Chapter 5** I use detailed reproductive data of six different plover populations belonging to five different closely related species with differing breeding systems and ASR to explore how the length of time spent in the breeding grounds (breeding schedule) by males and females may differ. Finally, **Chapter 6** summarizes the main research findings of this dissertation and discusses its contribution to the understanding of breeding system evolution under the light of the new models.

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2 Adult sex ratio and operational sex ratio exhibit different temporal dynamics in the wild

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Manuscript in press: Behavioural Ecology

This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.

Author contributions

MCC-I: study conception and design, data extraction and estimations, statistical analysis, manuscript preparation
SA: statistical advice, manuscript improvement
TS: study conception and design, manuscript improvement
APR-G: statistical advice, statistical analysis, manuscript improvement
MC-L: fieldwork, manuscript improvement
MAS-M: fieldwork, manuscript improvement
CK: study conception and design, field work, statistical advice, manuscript improvement

Abstract

Adult sex ratio (ASR, the proportion of adult males in the adult population) and operational sex ratio (OSR, the proportion of sexually active males in the mating pool) are fundamental properties of breeding populations and they are often linked to mating systems and sexual selection. However, ASR and OSR emerge via different routes in a population and may exhibit different temporal patterns. Here we use data from a well-monitored polygamous snowy plover *Charadrius nivosus* population sampled over three consecutive breeding seasons, to investigate whether temporal changes in ASR relate to changes in OSR. We found that snowy plovers exhibited male-biased ASR and OSR. Consistent with theoretical expectations, OSR was more variable than ASR. However, there was no consistent relationship between OSR and ASR: in only one out of three study years we found a weak positive relationship ($r = 0.22$). The lack of association was corroborated by time-series analyses and sensitivity tests. Our work therefore suggests that ASR and OSR exhibit different temporal patterns in a polygamous population, and we call for further theoretical and empirical studies that analyse their relationship across a range of different breeding systems.

Keywords: adult sex ratio, operational sex ratio, temporal variation, within-season variation, social environment, mating system

Introduction

Sex ratios are fundamental demographic properties of populations that are tightly linked to population growth (Bessa-Gomes et al., 2004; Donald, 2007; Veran and Beissinger, 2009), mating systems, parental behaviour (McNamara et al., 2000; Székely et al., 2000; Kokko and Jennions, 2008; Liker et al., 2014), and sexual selection (Emlen and Oring, 1977; Clutton-Brock and Vincent, 1991; Kvarnemo et al., 1995; Kvarnemo and Ahnesjö, 1996; Shuster and Wade, 2003; Silva et al., 2010). Sex ratios can be measured at several stages of development: at conception (primary), birth (secondary), and during adult life (adult sex ratio and operational sex ratio). Theoretical and empirical studies have identified numerous ecological and evolutionary implications of sex ratios during adult life across several taxa on ecology, behaviour and life histories (Kokko and Jennions, 2008; Veran and Beissinger, 2009; Székely et al., 2014).

In nature, sex ratios are highly variable and could change dynamically through time in a population (Emlen and Oring, 1977; Clutton-Brock and Vincent, 1991; Clutton-Brock and Parker, 1992; Kvarnemo and Ahnesjö, 1996; Forsgren et al., 2004). Nonetheless, studies that evaluate temporal variation in sex ratios are scarce (but see: Pettersson et al., 2004; Arendt et al., 2014) and it is unknown whether the variability of sex ratios is a common trait of all populations or associated with certain species, mating system or types of sex ratios.

Much attention has been paid to the demographic and life history mechanisms that generate biases in ASR: a) biases in early-life sex ratios (i.e. at conception or at birth) (Fisher, 1930; Wilson, 1975); b) sex-specific mortality of juveniles and/or adults (Clutton-Brock et al., 2002; Le Galliard et al., 2005); c) sex differences in maturation times (Lovich and Gibbons, 1990), d) sex-specific migration and dispersal (Dale, 2001; Clutton-Brock et al., 2002; Le Galliard et al., 2005; Steifetten and Dale, 2006) and e) sex differences in arrival time to breeding grounds (Rubolini et al., 2004; Kokko et al., 2006). Attention to how ASR impacts on behavioural and reproductive traits has increased recently (Donald, 2007; Kokko and Jennions, 2008; Székely et al., 2014). For example, male-biased ASR can be related to intensified aggression towards females in lizards (Le Galliard et al., 2005), influence mating rates in invertebrates and mammals (DeLong, 1978; Debuse et al., 1999; Karlsson et al., 2010), higher divorce rates in fish and birds (Beltran et al., 2009; Liker et al., 2014) and reversed sex roles in shorebirds (Liker et al., 2013).

Previous empirical and theoretical research on the importance of sex ratios for mating and parental care has focused primarily on OSR (Emlen & Oring 1977). For example, male-biased OSR is related to the development of sexual characters in insects (Pomfret and Knell, 2008),

loss of choosiness in male fish (Berglund, 1994), intensified courtship behaviour in fish (Forsgren et al., 2004; Silva et al., 2010) and higher female brood desertion rates in fish (Balshine-Earn and Earn, 1998). Theory suggests that the degree of monopolisation of mates may increase if OSR is biased towards one of the sexes leading to changes in the intensity of sexual selection within a population (Emlen and Oring, 1977; Kokko and Jennions, 2008; Jennions and Kokko, 2010). OSR emerges as a combination of variation in ASR plus the behavioural and mating decisions of individuals. As males and females in the adult population shift between sexually active and inactive periods dynamically (time-in and out, respectively; Clutton-Brock and Parker, 1992; Székely et al., 2000; Alonzo, 2010), OSR is expected to be more variable than ASR over time.

Predicting the relationship between OSR and ASR is not trivial. OSR and ASR may be tightly linked or correlated within a population, as the first is a subset of the latter and they have similar biological implications. But OSR and ASR are inherently different (Kokko and Jennions, 2008; Székely et al., 2014), and hence may be uncorrelated given that OSR may be more dynamic than ASR as it depends on the behavioural and mating decisions of individuals. A few studies have quantified and reported both OSR and ASR in wild populations at the same time (Whitfield, 1990; Mitani et al., 1996; Gerber, 2006; Veran and Beissinger, 2009; Chak et al., 2015). Nonetheless whether they are correlated with each other has not been formally tested. Although ASR and OSR may be different from each other and may not be correlated, several experimental studies use the concepts of OSR and ASR interchangeably (reviewed by Kokko and Jennions, 2008), perhaps because during initial experimental conditions when all individuals are unmated OSR will equal ASR if all the individuals involved are sexually active. Nevertheless, as the experiment progresses and individuals are allowed to interact, ASR will remain stable whereas OSR will inevitably change as some individuals mate and are in time-out and others re-enter the mating pool (Kvarnemo and Merilaita, 2006). ASR and OSR can only be identical or highly correlated, if periods of sexual activity are exactly the same or very similar in males and females, a condition that is rarely met in nature (Székely et al., 2014). Understanding the temporal relationship between ASR and OSR is an important precursor to understand the dynamics of mating competition and parental care.

We had two objectives in this study: first, to investigate the temporal variation of ASR and OSR, and second, to examine the correlation between ASR and OSR in a wild polygamous population. We studied the temporal variation in both ASR and OSR in a population of snowy plovers *Charadrius nivosus* (Küpper et al., 2009) breeding at Bahía de Ceuta, Mexico. This shorebird is suitable for exploring sex ratio variation for three reasons. First, snowy plovers are partial migrants, some spend only a part of the breeding season at the breeding site whereas

others remain in the area all year (CK & MC-L, unpublished data). Therefore, the date of arrival and departure to/from the breeding site may vary considerably between individuals, giving rise to temporal fluctuations in the number of adult males and females in the local population. Second, snowy plovers have variable breeding behaviour: both sexes incubate (Vincze et al., 2013) but after hatching, the female (or rarely the male) may abandon the brood. The remaining parent stays with the chicks until fledging. However, both parents often stay together until the offspring fledge (Warriner et al., 1986; Page et al., 2009). Third, snowy plovers are sequentially polygamous and often have multiple mates in one breeding season. Deserting parents attempt to quickly re-mate and start a new breeding attempt (Warriner et al., 1986; Beamonte-Barrientos et al. unpubl.). The caring parent may also re-nest with a new mate after successfully fledging the young. Taken together, the partial migratory behaviour and the complex mating system of snowy plovers may interact with both ASR and OSR. Because of the predominance of polyandry and male brood care in snowy plover populations, we predicted an overall male-biased ASR and OSR (Liker et al., 2013). We anticipated that OSR would have greater variation than ASR since it should change more dynamically over the breeding season than ASR. given that females normally abandon the broods and re-mate entering and exiting the breeding pool more frequently than males. Finally, because ASR and OSR have different dynamics and are influenced by different population parameters, we predicted that both indices are not correlated over time.

Methods

General fieldwork procedures

Fieldwork was carried out at Bahía de Ceuta, Sinaloa, Mexico (23º 54' N, 106º 57' W) during the snowy plover breeding season (April to July) from 2006 until 2011. Bahía de Ceuta is a wetland complex (1,497 ha) located at the Gulf of California, consisting on swamps, natural salt marshes and mangrove forests (Nava, 2007). The study area, a salt marsh surrounding several abandoned salt evaporation ponds, covers approximately 150 ha. Every year since 2006, this snowy plover population has been intensively monitored during the breeding season and typically 30-100 adult snowy plovers breed at the site (Carmona-Isunza et al., 2015).

We followed basic fieldwork methodology described in detail in Székely et al. (2008). We searched for nests by identifying incubating adults from a distance of 100-200 m using a mobile hide. We checked nests every 3-5 days to monitor clutch survival. We considered laying date as the date when the clutch was completed and parents started to incubate the

eggs persistently. If a nest with a complete clutch was found, we estimated clutch completion date by floating eggs in water when the nest failed (the density of eggs decreases progressively as they lose water during incubation) or from observed hatching dates assuming an incubation period of 25 days (Székely et al., 2008). We captured adults on the nest using funnel traps and marked them with a metal ring and with individual colour ring combinations (518 adults from 2006 until 2011). Around the expected hatching date, we checked nests daily in order to ring chicks before they left the vicinity of the nest. In addition to the metal ring, we marked chicks with a single colour ring to help identification in the field. Some families were only encountered for the first time after the chicks had left the nest. For those families, we estimated chick age and hatching date (and thereafter laying date) using the tarsus length of chicks, assuming linear growth (Dos Remedios et al., 2015). We considered a brood as fledged and independent when the oldest chick had reached the age of 25 days old (Székely and Cuthill, 1999).

We recorded the number of chicks and the sex of the attending parent(s) immediately after chicks hatched and repeated these observations every 2-4 days until the brood had perished or fledged. When only one parent was present, we observed the focal brood for at least 15 min or until the missing parent was seen. This allowed us to make sure that missing parents were not hiding or only temporarily absent. If the same parent was absent during two encounters in a row (without re-appearing in subsequent observations) we considered the parent as having abandoned the brood. Additionally, we recorded the identity and sex of every ringed individual that was re-sighted throughout the breeding season using a spotting scope or binoculars, noting the date, time and location.

Population surveys

We carried out population surveys every two weeks during the breeding season and once a month or every two months outside of the breeding season during 2009, 2010 and 2011. During each survey, observers moved slowly with a mobile hide through the entire study area and stopped every 100-200 m to record colour ringed plovers, the number of unmarked plovers and the sex of each adult (ringed or unmarked). Sexes were identified according to the dimorphic plumage present during the breeding season: plover males have darker head and breast bands than females (Argüelles-Ticó et al., 2015) and confirmed by molecular sexing (details in Dos Remedios et al., 2015). All colour ring combinations recorded on each survey were subsequently checked using the record of colour ring combinations used in this population; observations with incomplete or inexistent ring combinations were considered erroneous readings and were not used in the analyses. This is justified, since colour ringing of snowy plovers at other locations nearby (<2000 km) started only in 2010, and only one

individual ringed elsewhere was observed in 2012 among a total of 1678 sightings of colour ringed individuals since 2009 (CK & MC-L, unpublished data). Therefore the chances of encountering a ringed individual from another location are small. In 23 (0.014%) cases the sex of ringed individuals recorded during surveys did not match the sex registered on previous captures; in these few cases, the sex recorded during surveys was replaced with the presumably correct previously determined sex when available.

Estimating individual presence intervals

Not all snowy plovers remain at the breeding site throughout the entire breeding season. Breeding dispersal is high and males and females may nest at different locations hundreds of kilometres apart (Stenzel et al., 1994). Therefore, we estimated the presence intervals of individuals (periods of time when individuals were present) in the population during each breeding season based on information from nesting, family observations and presence in surveys. We defined the start of a breeding season as 10 days before the first laying date recorded in the study site each year, midpoint of the 8-11 days it took individuals to bond with a partner and establish a nesting territory (see ***Estimating time-out periods***). We considered a breeding season finished 25 days after the last brood hatched in the population each year, the minimum time in which plover chicks fledged (normally 25-30 days after hatching, Székely and Cuthill, 1999). We used records from breeding-season surveys and non-breeding season surveys that were conducted a maximum of 30 days before the start or after the end of the breeding season, opportunistic re-sightings, nest and brood checking to determine the day when each individual was first seen (hereafter “arrival date”), and the day when it was last seen (hereafter “departure date”, see Fig. 1a for examples). We assumed that each individual remained at the study site for the entire presence interval delimited by its arrival and departure date.

Estimating ASR

ASR estimates were based on presence intervals of a sample of breeding adults. Our sample consisted of 111 ringed adults (in 2009, 2010 and 2011, males: 46, 38, 24; females: 27, 32, 21 respectively) that bred in at least one of the three years of the study, and were individually ringed at least one year before the focal year (i.e. known individuals). Ringing takes place during incubation and breeders ringed in a focal year would have been recorded in surveys or re-sightings only after their ringing date. The sample used represented the 71% (2009), 54% (2010) and 48% (2011) of the total number of ringed breeders each year and we only calculated presence intervals for these known individuals. To estimate ASR at any given day of the breeding season, we counted the number of known males and females present that day

(using the estimated presence intervals, see examples of this computation in Fig. 1b). ASR was computed as the number of males present divided by the total number of males and females present.

Estimating time-out periods

A time-out period is the time spent during pair-bonding and caring for eggs or chicks, i.e. when an individual is "out" of the breeding pool, following the definition of Clutton-Brock and Parker (1992). We calculated time-out periods for each reproductive event of each ringed individual; individuals were considered as available to breed ('time-in') during the periods when they were not in time-out (Fig. 1a), i.e. the conditions are mutually exclusive. For each time-out period, we calculated its initial day (T_i) and final day (T_f). T_i was defined as the initial day of pair-bonding characterised by courtship, nest scraping and/or copulation behaviour. Actual dates when pair-bonding started were unknown, therefore T_i was estimated as the date when the last egg of the clutch was laid minus 10 days (Fig. 1a). We estimated this 10 day-period of pair-bonding using the mean time it took for re-nesting individuals to complete a new clutch (mean ± s.e.: 9.37 ± 0.89 days, n = 22 males and 49 females that re-nested).

T_f coincided with nest (or brood) failure, or the termination of parental care. For failed nests (e.g. flooded, eggs predated or disappeared), T_f of males and females was considered to be the day when the nest was found with no eggs; when this information was not available, T_f was the date when the last egg of a failed clutch was laid (Fig. 1a (a)). For nests that produced at least one chick, T_f depended on whether the parents deserted their broods and on chick survival. For a deserting parent, T_f was the midpoint between the last day the parent was seen caring for its brood and the first day it was absent (Fig. 1a (b)). For caring parents, T_f depended on chick survival in two aspects: (i) if all chicks died before fledging, T_f was the midpoint between the last date when the last surviving chick was seen alive and the first day when the parent was seen without the chick(s) (Fig. 1a (c)); (ii) if chick(s) fledged, T_f equalled the hatching date plus 25 days, the time required for the offspring to fledge and make the transition to independence (Székely and Cuthill, 1999) (Fig. 1a (d)). Whenever the survival of chicks was unknown, T_f was the last day when the family was seen, except for chicks that were still alive when fieldwork was concluded; in the latter case chicks were considered to have fledged.

We assumed an individual was in the breeding pool whenever T_f from its last breeding event and T_i from the next breeding event did not overlap. This includes also cases when an

individual did not switch mates because we reasoned that the opportunity to change mates existed.

Estimating OSR

To estimate OSR, we used the same sample of ringed adults used to estimate ASR (described above). We counted the number of known males and females present that were available to breed (i.e., they were not in time-out, see examples of this computation in Fig. 1b) at any given day of the breeding season. Similar as ASR, OSR was computed as the number of males present and available to breed divided by the total number of males and females present and available to breed in our sample. For days when we could not compute OSR (i.e. division by zero because all marked adults were unavailable for breeding, 6.6% of days all recorded in the beginning of the breeding season of 2011) we set OSR to zero for analysis and omitted these records from the figures. Omitting these few cases from the analysis or replacing its value for the mean OSR did not change the results.

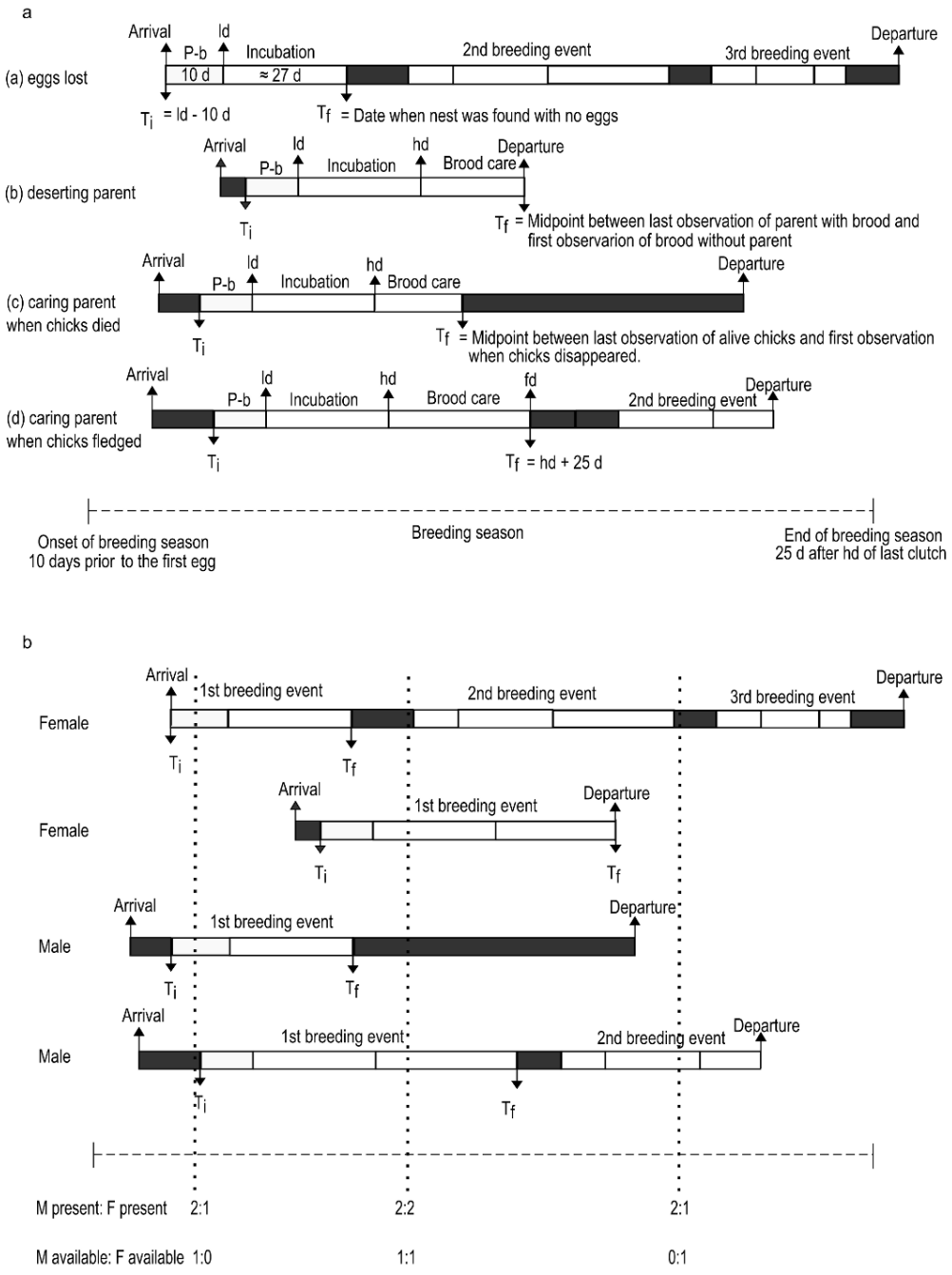

Fig. 1a Time-out periods (white boxes) of male and female snowy plovers for: (a) failed nesting attempts, (b) deserting parents, (c) caring parents when chicks died, and (d) caring parents when chicks fledged. Examples of arrival and departure dates are shown, and filled boxes represent examples of the period of time an individual was available to breed (time-in), *ld* stands for laying date, *hd* for hatching date, *fd* for fledging date, T_i is initial date of time-out, T_f is final date of time-out. Second and third breeding events may have occurred for some individuals as examples show, these events are split in three periods corresponding to pair-bonding time (*P-b*), incubation and brood care. **b** Example that shows how ASR and OSR were calculated. Vertical dotted lines represent example days for which the number of males (*m*) and females (*f*) were counted using the dates of arrival and departure and the estimated time-out periods.

Statistical analyses

In order to describe temporal variation in ASR and OSR we report time-series plots showing daily estimates with their associated 95% confidence intervals for proportions (CIs) calculated using the Clopper and Pearson method (Clopper and Pearson, 1934), and provide the estimated median and interquantile ranges of ASR and OSR per year. We used Levene's test (based on absolute deviations from the median) to compare variation between ASR and OSR.

Daily estimates of ASR and OSR (estimates for a total of 119 (2009), 131 (2010) and 115 days (2011)) are non-independent data points obtained over a time interval, therefore, in order to test whether ASR and OSR were correlated we performed a formal time series analysis that started by removing the trends of the series to avoid spurious correlations, as suggested by Box and Jenkins (Chatfield, 2003). We carried out time-series analyses separately for each year and for ASR and OSR. We removed the trends present in the series by using one degree differentiation (i.e. computing differences between consecutive observations) (Chatfield, 2003). We confirmed non-stationarity of differenced series using the unit root test, but also used periodograms and autocorrelation plots to ensure the series were detrended. Despite the differentiation, the ASR estimates of 2011 and OSR estimates of 2009, still suffered from autocorrelation, thus, for these two series, autoregressive moving average models (ARIMA) were fitted applying the Box-Jenkins approach (Chatfield, 2003). To cross-correlate ASR and OSR separately for each year and examine their association we used the differenced series and residuals from the ARIMA models to evaluate the non-autocorrelated part of each series, i.e. the stochastic term.

Cross-correlations allowed us to test the correlation of ASR and OSR on the same point in the time series (zero day lag, corresponding to a Pearson correlation) but also the correlations for various time lags. We performed cross-correlations for -20 to 20 day lags to examine the potential lagged relationship between ASR and OSR. We only report cross-correlation coefficients for zero day lag to describe the correlation between ASR and OSR on a same day, and cross-correlation coefficients for the peak cross-correlation observed in each year throughout all lags to evaluate potential cross-correlations in lagged series.

Sensitivity analysis

The estimation of ASR and OSR in this study was not computed from direct counts of males and females in a census; instead it was derived from data on reproduction collected for each individual. The data of reproductive events is inherently based on simplified assumptions (e.g. chicks fledge after 25 days), estimations (e.g. chicks age calculated with tarsus and beak lengths) and field observations (e.g. colour codes recorded by several observers) that

introduced errors or variation that the method did not account for. We used two parameters to estimate ASR, arrival date and departure date (Table 1), whereas to estimate OSR, we used arrival date and departure date, plus three other parameters: laying date, 10 days of pair-bonding (to estimate T_i), and T_f (Table 1). Error or variation in these five parameters could potentially lead to spurious biases in ASR and OSR.

To explore how variation/error in these parameters could influence our baseline values of ASR, OSR and their correlation we carried out a sensitivity analysis in three steps. First, we modified each parameter of each individual by drawing a new date at random from a normal distribution having a mean equal to the original estimation of the parameter of that specific individual and a standard deviation of ± 1 day. Second, using the new dates of parameters generated for each individual we estimated ASR and OSR newly, using only one of the modified parameters at a time. Parameter modification yielded two different estimations for ASR (Table 1 a-b) and four different estimations for OSR (Table 1 a-d). We also report an additional estimation for ASR and OSR in which all parameters were modified at the same time (Table 1e). Finally, we ran time-series analysis (as described above) with each new estimate of ASR and OSR and ran cross-correlations between all possible combinations of the different estimates of ASR and OSR.

Statistical analyses and plots were carried out using R (R Development Core Team 2015, Version 3.2.0). Time-series analysis and cross-correlations (*crosscorr* in Econometrics Toolbox) were performed using Matlab (R2012b, The MathWorks Inc., Natick, MA, 2012).

Table 1. Definition of parameters used to estimate adult sex ratio (ASR) and operational sex ratio (OSR) in the analyses of snowy plover data. The table also provides the parameter values we used in sensitivity analyses.

Parameter	Baseline parameters definition	Modified parameters per individual*	Sex ratio impacted	
			ASR	OSR
a) Arrival date	First time seen	First time seen ± 1 d	✓	✓
b) Departure date	Last time seen	Last time seen ± 1 d	✓	✓
c) T_i	Laying date minus 10 days of pair bonding	T_i ± 1 d	-	✓
d) T_f	T_f day when the nest was found with no eggs, parent deserted, chicks died or fledged	T_f of individual ± 1 d	-	✓
e) All parameters modified at once		arrival date of individual ± 1 d, departure date of individual ± 1 d, laying date of individual ± 1 d, 10 days of pair bonding ± 1 d, T_f of individual ± 1 d	✓	✓

***** T_i (initial date of time-out), T_f (final date of time-out), d (day); modified parameters were randomly drawn for each individual in our sample from a normal distribution with the mean ± sd specified in the table

Results

Temporal variation in ASR and OSR

Both ASR and OSR tended to be male-biased (ASR median = 0.64, 0.56, 0.60; OSR median = 0.75, 0.59, 0.75; n = 119, 131, 115 days in 2009, 2010 and 2011, respectively), although in daily estimates the majority of 95% confidence intervals included 0.5 (Fig. 2). In general, confidence intervals of OSR were larger than those for ASR. OSR interquartile ranges were substantially larger than those of ASR throughout the three years (interquartile ranges of ASR: 0.02, 0.05, 0.09 and OSR: 0.13, 0.21, 0.31 in years 2009, 2010 and 2011 respectively). Consistently, the variance in OSR was significantly more extensive than that of ASR (Levene's test: $F_{1,714}$= 221.75, P < 0.01).

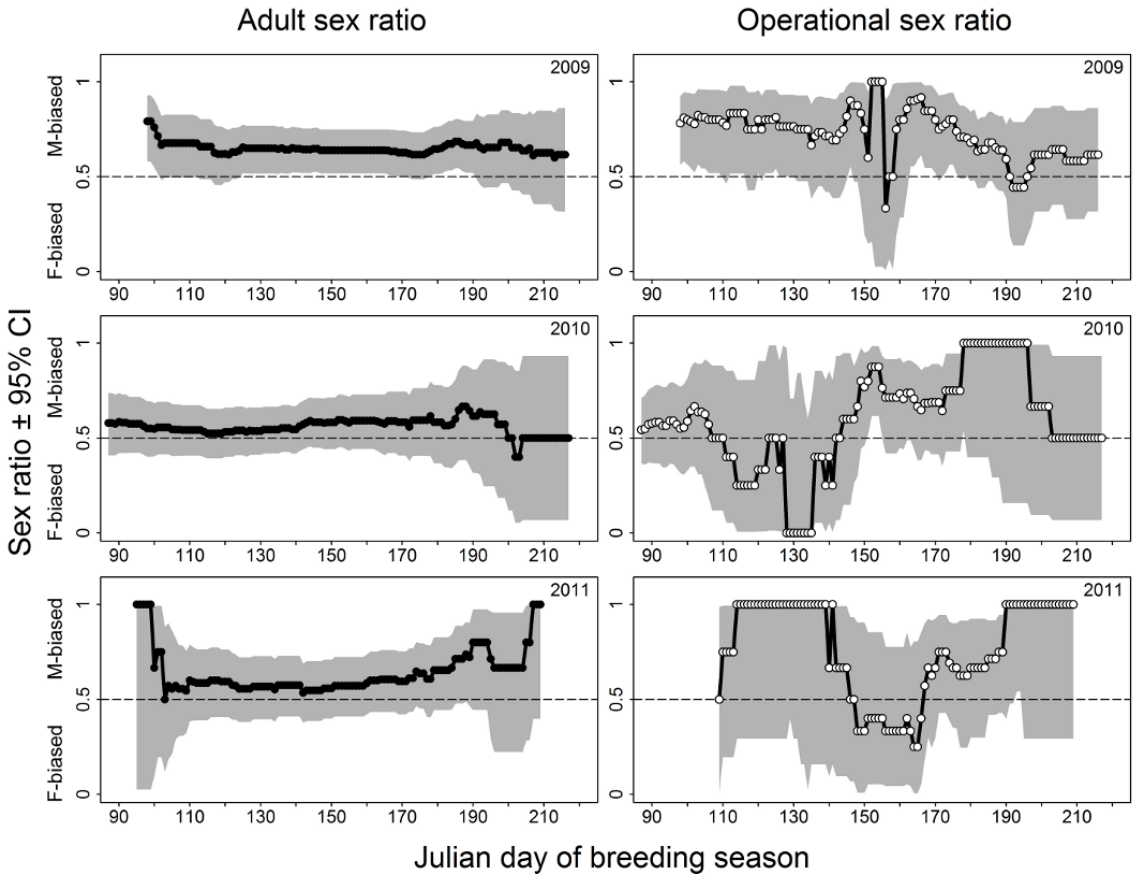

Fig. 2 Adult sex ratio (ASR, filled dots) and operational sex ratio (OSR, open dots) time series with 95% confidence intervals in snowy plovers breeding at Bahía de Ceuta, Mexico. Broken line demarks unbiased sex ratio (1:1). Julian day refers to the number of days since the 1st of January.

Does ASR predict OSR?

Same day correlations (zero day lag) of ASR and OSR varied each year, in 2009 it was negative but non-significant; in 2010 positive and significant and in 2011 it was positive but non-significant (Table 2). These results were consistent with those derived by peak cross-correlations: the peak correlation in 2009 was non-significant (peak cross-correlation: r = 0.13, lag = -15 days, *P* = 0.53; Fig. 3). In 2010, the peak correlation corresponded to the zero day lag indicating that more male-biased ASRs were correlated with more male-biased OSRs instantaneously (peak cross-correlation: r = 0.22 , lag = 0, *P* = 0.01; Table 2: Original ASR versus Original OSR, Fig. 3). Year 2011 showed a negative peak correlation (r = -0.35, lag = 9 days, *P* = 0.001; Fig. 3), i.e.male-biased ASRs were correlated with female-biased OSRs (and *vice versa*) 9 days later.

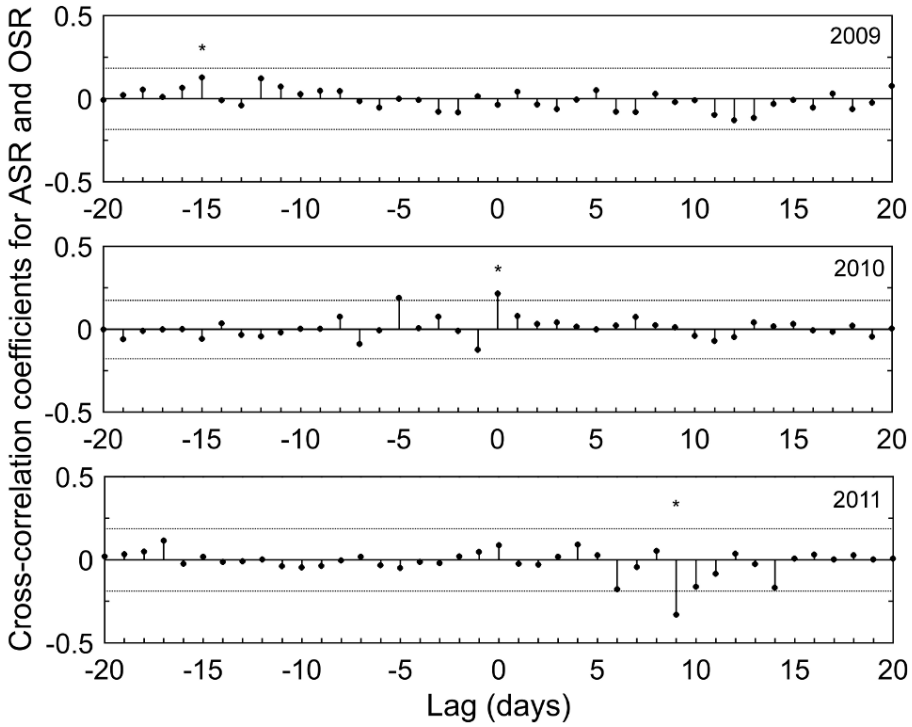

Fig. 3 Correlation coefficients of cross-correlation analysis between time series of adult sex ratio (ASR) and operational sex ratio (OSR) of snowy plovers during three years of study. The x-axis indicates the number of days by which ASR leads (- lags) or lags (+ lags) OSR. Horizontal dotted lines show approximate upper and lower confidence bounds of cross-correlations assuming ASR and OSR are uncorrelated. The asterisks show peak correlations in each year.

Table 2. Adult sex ratio (ASR) and operational sex ratio (OSR) cross-correlation coefficients for zero day lag when parameters for calculating sex ratios were modified for the sensitivity analysis (see Table 1). Correlation coefficient with *P*-values in parenthesis are shown for all possible correlations per year, significant correlations are shown in bold.

ASR \ OSR	Year	Original parameters	Arrival date	Departure date	T_i	T_f	All parameters
Original parameters	2009	-0.03 (0.70)	-0.01 (0.88)	0.04 (0.67)	0.02 (0.81)	-0.007 (0.94)	0.001 (0.98)
	2010	**0.22 (0.01)**	**0.21 (0.02)**	0.16 (0.06)	**0.22 (0.01)**	**0.18 (0.04)**	0.13 (0.15)
	2011	0.09 (0.36)	0.13 (0.15)	**-0.30 (<0.001)**	**-0.28 (<0.01)**	0.10 (0.26)	-0.06 (0.51)
Arrival date	2009	0.04 (0.65)	-0.02 (0.79)	0.10 (0.26)	0.09 (0.35)	0.04 (0.70)	0.01 (0.93)
	2010	**0.20 (0.03)**	**0.20 (0.02)**	0.13 (0.14)	**0.19 (0.03)**	0.16 (0.07)	0.14 (0.11)
	2011	0.02 (0.83)	0.03 (0.73)	**-0.52 (<0.001)**	**-0.52 (<0.001)**	0.03 (0.75)	0.02 (0.81)
Departure date	2009	0.04 (0.64)	-0.02 (0.80)	0.10 (0.26)	0.08 (0.34)	0.03 (0.70)	0.01 (0.92)
	2010	**0.20 (0.03)**	**0.20 (0.02)**	0.13 (0.14)	**0.19 (0.03)**	0.16 (0.07)	0.14 (0.11)
	2011	0.02 (0.83)	0.03 (0.73)	**0.53 (<0.001)**	**-0.52 (<0.001)**	0.03 (0.75)	0.02 (0.80)
All parameters	2009	0.15 (0.08)	-0.04 (0.69)	0.04 (0.68)	0.04 (0.62)	0.02 (0.93)	-0.03 (0.78)
	2010	0.15 (0.08)	0.14 (0.11)	**0.22 (0.01)**	0.13 (0.13)	0.12 (0.16)	**0.22 (0.01)**
	2011	0.02 (0.99)	0.02 (0.83)	**-0.52 (<0.001)**	**-0.53 (<0.001)**	0.01 (0.95)	0.03 (0.71)

Sensitivity analyses

The correlations between ASR and OSR were sensitive to systematic changes in the five parameters we used to compute these ratios parameter values. In 2009 none of the parameter values resulted in a significant association between ASR and OSR (Table 2). In 2010 the significant positive correlation observed between the baseline values of ASR and OSR persisted for 13 out of 24 parameter values, the rest were positive but non-significant (Table 2). In 2011 the positive but non-significant relationship between ASR and OSR originally observed with the baseline values, became significantly negative at 8 out of 24 parameter values. When all parameters were altered simultaneously for ASR and OSR, the observed association between them at a zero day lag was consistent with the baseline association for all three years.

Peak cross-correlations for the different modified parameter values varied in direction and lag each year (results not shown). In 2009 no peak cross-correlations observed were significant. In 2010 peak cross-correlations were significant in 15 out of 24 parameter values (14 positive, one negative; lags between -12 and zero). In 2011 all 24 peak cross-correlations were significant (nine positive, 15 negative with lags between 0 and 14).

Discussion

By estimating temporal variation in both ASR and OSR in a wild bird population, our work provided two major results. First, ASR and OSR were both male-biased, and OSR showed greater temporal variation than ASR. Second, cross-correlation tests failed to detect a consistent relationship between ASR and OSR: in two study years (2009 and 2011) ASR and OSR were uncorrelated, whereas in one study year (2010) these ratios showed as small positive correlation. Additionally, the sensitivity analysis showed that slight changes in the parameters used to estimate ASR and OSR had large impacts on their correlation coefficients.

Bias and temporal variation of sex ratios

Male-biased ASR in the snowy plover's Ceuta population is consistent with male-biased ASR reported in other populations of snowy plovers (Stenzel et al., 2011) and with generally male-biased ASRs reported in birds (Donald, 2007; Pipoly et al., 2015). In birds, higher mortality in females than males (Promislow et al., 1992; Liker and Székely, 2005) is the likely cause for male-biased ASR (Gerlach and Le Maitre, 2001; Githiru and Lens, 2006; Székely et al., 2006; Kosztolányi et al., 2011). Biases can be reinforced by higher dispersal and/or further migration of females (Greenwood and Harvey, 1982; Kjellen, 1994; Clarke et al.,

1997; Dale, 2001; Stouffer et al., 2003; Catry et al., 2005; Steifetten and Dale, 2006) or by sex differences in time of arrival to the breeding grounds (Clutton-Brock and Parker, 1992; Payne et al., 2011). Male-biased ASRs observed in local populations could also be a consequence of higher mobility of one sex during the breeding season (Küpper et al., 2012) coinciding with a higher turnover and shorter periods spent at a given breeding site by females than males. Consistent with this, snowy plover females spent less time in Ceuta than males (mean throughout the three years, females: 81.31 ± 4.5 days, n = 83 females; males: 93.72 ± 3.32 days, n = 110 males, $t_{159} = -2.22$, $P = 0.03$). If females have higher mobility than males, more unringed females than males could visit the breeding site for breeding reducing the true sex ratio bias. However, we did not find differences between the numbers of newly ringed males and females across the study years (males: 15, 35, 22; females: 21, 27, 26; $\chi^2_2 = 2.34$, $P = 0.31$, n = 3 years).

Similarly to ASR, median OSR was also male-biased in Ceuta. In comparison with ASR, daily estimates of OSR showed higher temporal variation throughout the breeding seasons as shown by larger interquartile ranges of OSR compared to ASR using a conservative test. This is consistent with theoretical models that suggest that OSR is a dynamic outcome of mating and parental decisions (Kokko and Jennions, 2008), whereas ASR is largely influenced by demographic processes and therefore should vary less (Székely et al., 2014). Given the strong variation in OSR with shifts from strong male-bias to strong female-bias initially expected, we had predicted the overall OSR to be slightly male-biased or unbiased. However, we found a strong male-bias in OSR. This may be because periods with strong female-bias were scarce and brief, implying either that females in the breeding pool re-mate very quickly or only stay at the site for brief periods of time.

The biases of both ASR and OSR are conservative since both estimates were determined using the previously marked part of the population only. This is a consequence of our field methods because most adults are trapped on the nest and therefore must have bred at least once at the site previously. Since polyandrous plover females have higher mating opportunities than males (Székely et al., 1999), it is plausible that a larger proportion of males than females remained unmarked and therefore the sex ratio bias towards males could be even stronger as suggested by demographic models in other polyandrous plover populations (Kosztolányi et al., 2011).

We are aware of only two studies in a single species analysing temporal variation in ASR throughout the breeding season. On these studies, ASR was highly variable throughout the year and markedly different between populations of the Trinidadian guppy (Pettersson et al.,

2004; Arendt et al., 2014), as some populations exhibit an overall male-biased or female-biased ASR, or no bias at all (Pettersson et al., 2004). Trinidadian guppies present a complex mating system: females are overall promiscuous but males may use either a courting or sneaking tactic to mate with females (Houde, 1997) depending on environmental factors such as light (Chapman et al., 2009), food availability or parasites (Kolluru et al., 2009). However, given that ASR is a strong predictor of mating system in shorebirds (Liker et al., 2013), it will be interesting to explore if the variability in ASR will also explain the complex and varying mating system in guppies. By contrast, snowy plover populations are consistently polyandrous and exhibit ASRs that are more stable in time and consistently male-biased throughout populations (this study; Warriner et al., 1986; Stenzel et al., 2011; Carmona-Isunza et al., 2015). Consistency of the sex ratio bias might be negatively correlated to the flexibility of the mating behaviour. For example, mating systems might show little flexibility in species where ASRs remain stable through time, whereas populations with variable mating systems may exhibit more variable ASRs. Comparative phylogenetic studies are needed to test if this is a widespread pattern.

Correlation between ASR and OSR

There was no consistent relationship between ASR and OSR. We found a weak correlation in one year and no correlation in the other two years. Unpublished population estimates of ASR and OSR indicate that the correlation in other bird and mammal populations may be positive (~0.4-0.5, Liker pers. comm. using data from Whitfield, 1990; Mitani et al., 1996). However, since theory indicates that OSR is more labile than ASR (Kokko and Jennions, 2008) a consistent relationship is not necessarily expected.

Diverse mating systems may influence the relation between ASR and OSR. For example, ASR and OSR are expected to be correlated in monogamous populations where males and females have similar time-in and time-out periods. In contrast, in polygamous populations with sex difference in the extent of care, the time-in and time-out of males and females may differ, and this difference in turn, lead to weak (or no) relationship between ASR and OSR. For example, birds tend to have male-biased ASRs, and since they exhibit social monogamy and biparental care of the young, the male-biased ASR is expected to translate into male-biased OSR (Donald, 2007), yielding a potentially strong correlation between them. It is important to note that in species where extra-pair paternity occurs as a mating strategy such as many passerine birds establishing female time-out and time-in periods is not trivial. Contrastingly, in mammals which tend to exhibit female-biased ASR, the OSR may still be male-biased given that parental care is largely provided by the females(Székely et al., 2014)

and this may translate into a potentially poor correlation between ASR and OSR. It will be interesting to undertake multi-population studies evaluating ASR and OSR in populations with different mating systems. Taxa with high variation in mating system like fish, frogs and other avian species pose good models to indagate differences in how ASR and OSR relate to each other.

We observed a high stochasticity in the relationship between ASR and OSR. This may be the result of environmental fluctuations (e.g. climatic) and/or density-dependent processes (e.g. competition for territories or food) that impact population dynamics, physiology and breeding activity of individuals. Fluctuations observed in correlations of ASR and OSR throughout the years may be related to the constraints of our data set. The number and proportion of previously marked breeders in our sample decreased in each year (see Methods). The sex ratios of marked and unmarked plovers may have been different, if trapping probability is altered by sex-specific mobility and breeding activity. This may have resulted in a lower accuracy for estimations particularly in 2010 and 2011 in comparison with 2009. In line with this argument the sensitivity analysis changed the result of the correlation test less dramatically in 2009 than in the other two years. Further studies are needed to understand to what extent these fluctuations and density-dependent processes interact to produce variation in observed sex ratios.

Lagged correlation between ASR and OSR

There was no consistent time lag for peak correlations between OSR and ASR. A negative peak correlation coefficient is predicted if the male bias in ASR is positively related to female brood desertion which affects the OSR several days later. This is a plausible explanation as it is expected that if re-mating opportunities of females increase, females adjust their degree of involvement in parental care (Kokko and Jennions, 2008). However, we observed this only vagely in one of the three years whereas the mating and parental care patterns did not differ substantially across the three years (CK & MC-L, unpublished data).

ASR and OSR estimation accuracy

Estimating ASRs in wild populations is challenging as behavioural and ecological differences between males and females can make one sex more conspicuous than the other and can give rise to additional biases in ASR estimates (Donald, 2007; Székely et al., 2014). ASR estimates based on capture-mark-recapture methods can deal with sex differences in detectabilities, at least partially (Veran and Beissinger, 2009; Pickett et al., 2012), but these procedures are not always feasible since the models involved require detailed estimates of a range of demographic parameters for males and females.

Estimating OSR in wild populations is similarly challenging because the reproductive availability of an organism is rarely known. There is no formal consensus on how OSR should be estimated in wild populations and therefore several methods have been used to estimate it. OSR has been estimated during surveys where the observer establishes if the individual is paired or provides parental care to decide if it is available to breed or not (e.g. Whitfield, 1990). Other studies follow the methodology of Clutton-Brock and Parker (1992) and estimate OSR using population estimates of time-out periods of males and females and ASR estimations (e.g. Mitani et al., 1996). However, the latter approach allows only to know a single OSR estimate per breeding season, and therefore is not suitable to capture the temporal variation that exists. We argue that although OSR may be a useful indicator of breeding opportunities, it cannot replace ASR for three reasons: (1) it is not trivial to judge whether an individual is sexually active or not, (2) we demonstrated that it is less stable in time than ASR and (3) we also showed that OSR-biases do not necessarily reflect ASR-biases, at least in a polygamous population.

In this study we combined survey data with highly resolved information about the apparent breeding status of individuals to estimate ASR and OSR at the same time. This approach addressed differences in male and female detectability and allowed us to study intensively ASR and OSR variation over time periods. Importantly, our sensitivity analysis showed that a small change in the estimates for arrival, departure and time-out periods alters the observed relationship betweenASR and OSR dramatically. Sensitivity analyses as employed here can help to deal with uncertainties of the breeding status of an individual, especially in polygamous and promiscuous populations where individuals might be paired but still seeking copulations.

In conclusion, we found no consistent relationship between ASR and OSR in a polygamouswild bird population across multiple years. Estimating variation in sex ratios remains challenging but its estimation is essential to understand the dynamics of mating and parental care systems as comparative studies suggest that breeding behaviour and sex ratios are tightly linked (Liker et al., 2013; Liker et al., 2015). We argue that more parallel estimates of ASR and OSR in wild populations are necessary for comparative and multi-population studies that examine the influence of sex ratios on social behaviour and their relationship under different mating system contexts.

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Data accessibility

Analyses reported in this article can be reproduced using the data provided by Carmona-Isunza et al. (2016).

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3 Courtship behaviour differs between monogamous and polygamous plovers

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This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.

Author contributions

MCC-I: study conception and design, data extraction and estimations, statistical analysis, manuscript preparation
CK: fieldwork support, manuscript improvement
MAS-M: fieldwork support, manuscript improvement
TS: study conception and design, statistical advice, manuscript preparation

Abstract

Courting, accessing and/or competing for mates are involved in sexual selection by generating differences in mating success. Although courtship behavior should reflect intensity of mating competition and sexual selection, studies that compare courtship behavior across populations/species with different mating systems subject to differing degrees of mating competition are scanty. Here we compare courtship behavior between two closely related plover species (*Charadrius spp*): a polygamous population of snowy plovers and a socially monogamous population of Kentish plovers. Consistently with expectations, both males and females spent more time courting in the polygamous plover than in the monogamous one. In addition, courtship behavior of males relative to females increased over the breeding season in the polygamous plover whereas it did not change in the monogamous one. Our results therefore suggest that courtship behavior is a fine-tuned and informative indicator of sexual selection in nature.

Keywords: sexual selection, courtship behavior, mating behavior, mating systems, monogamy, polygamy, within-season variation

Introduction

Mating systems are associated with the sexual selection acting on males and females (Andersson 1994; Shuster and Wade 2003; Jennions and Kokko 2010; Kokko et al. 2012; Fritzsche and Arnqvist 2013). Sexual selection is expected to be stronger in polygamous populations since variation in mating success among individuals of the sex that competes more intensively for mates tends to be higher than in monogamous populations. The relationship between sexual selection and mating competition is revealed by theoretical and comparative studies that show sex-role reversal, increased sexual dimorphism in size, weaponry and/or ornamentation in polygamous taxa (Møller and Pomiankowski 1993; Székely et al. 2000; Pérez-Barbería et al. 2002; Wilson et al. 2003; Fairbairn et al. 2007; Rosenqvist and Berglund 2011). However, not only secondary sexual characters such as ornaments and armaments can influence reproductive success, but also behaviors that help individuals enhance their access to mates, such as courtship displays. Numerous studies have shown that courtship displays are variable across species and populations and have attributed this variation to differences in their morphology or habitat, or to avoid hybridization in sympatric species (van den Assem and Werren 1994; Hankison and Ptacek 2007; Quinn and Hews 2010; Pedroso et al. 2013; Wang et al. 2015), but there is limited information on whether differences in courtship behavior are associated with differences in their mating systems (but see Hollis and Kawecki 2014; Parra et al. 2014).

The strength of mating competition, and therefore sexual selection, may exhibit temporal variation in natural populations (Grant and Grant 2002; Kasumovic et al. 2008; Siepielski et al. 2009). A common predictor of this variation is the operational sex ratio (OSR), i.e. the ratio of sexually active males to females (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996), which may fluctuate dynamically throughout the breeding season (Forsgren et al. 2004; Kasumovic et al. 2008). If OSR is biased towards one sex, more intense sexual selection among the overrepresented sex is expected. Courtship behavior is labile and it can be expected to vary dynamically in response to shifts in mating competition and mating opportunities (Kokko et al. 2012; Parra et al. 2014) associated with fluctuations in OSR or other ecological variables. For example, in a wild population of two-spotted gobies (*Gobiusculus flavescens*) males court actively early in the season when OSR is highly male-biased, while females court more actively than males late in the season, when OSR is female-biased (Forsgren et al. 2004; Myhre et al. 2012). Few studies have examined how OSR variation relates to courtship within a single population and understanding how courtship behavior responds to temporal variations in sexual selection in populations or

species with different mating systems may contribute significantly to understanding the dynamics of sexual selection in nature.

Here we compare the proportion of time spent courting in two wild populations of closely related species: polygamous snowy plovers (*Charadrius nivosus*) in Ceuta, Sinaloa, Mexico, and monogamous Kentish plovers (*Charadrius alexandrinus*) in Maio, Cape Verde. Behavioral comparisons between these two species are feasible for three reasons: (1) snowy plovers and Kentish plovers were considered a single species until recent reclassification based on molecular evidence (Küpper et al. 2009; Dos Remedios et al. 2015), (2) our group has carried out a long-term monitoring on both sites, and (3) both sites are ecologically similar as they are natural salt marshes in tropical regions with mean annual temperatures between 23-25°C. Importantly, both species share similar life histories, behavior and ecology as both are non-colonial ground nesting insectivorous birds, but mating system may vary across different populations (Page et al. 2009). Snowy plover populations are generally polygamous as males and females (usually more than males) may re-mate with different mates each breeding season (Warriner et al. 1986). Some Kentish plover populations are polygamous, for example in France, Spain and United Arab Emirates (Lessells 1984; Amat et al. 1999; Kosztolányi et al. 2009), while others are monogamous for example in Saudi Arabia and Cape Verde (in Maio over 90% of adults keep their mate between successive breeding events; O. Vincze et al. unpubl. data). Therefore variation in mating system of plovers might be attributed to factors that vary throughout populations, rather than to differences between species. Despite having contrasting mating systems, both species' populations studied present low extra-pair paternity rates (below 5%; K. Maher et al. unpubl. data).

We analyzed behavioral observations from snowy plovers and Kentish plovers collected during their peak breeding season. We predicted that (1) males and females from the polygamous snowy plover population would spend a higher proportion of time courting than males and females from the monogamous Kentish plover population; (2) females would court more than males (i.e., courtship bias) in the polygamous snowy plover population, since females present a larger frequent mate change compared to males, whereas no courtship bias was expected in the monogamous Kentish plover population. Since previous studies found variation in courtship behavior and mating in relation to time in the breeding season (Székely et al. 1999; Forsgren et al. 2004), we also investigated whether courtship behavior varied within the breeding season.

Methods

Study species and study sites

We studied snowy plovers between April and May 2014 at Bahía de Ceuta, México (23º54' N, 106º57' W) where the annual peak breeding activity occurs from April till June. We studied the Kentish plovers between September and November 2013 at Maio, Cape Verde (15° 08' N, 23° 13' W) where the annual peak breeding activity occurs from September till December. Approximately 30-100 pairs in Ceuta and 100-200 pairs in Maio breed every year around saline lake areas and saltpans ranging from 120 to 382 ha surrounded by mangrove (Ceuta) or sandy shores (Maio). Individuals from both species stay all year round at the study sites (TS pers. obs.), although Ceuta hosts migratory and resident snowy plovers. In both species males and females incubate 2-3 eggs for 22-27 days (Vincze et al. 2013), but parental care differs: in the snowy plover, one parent (usually the female) abandons the brood shortly after hatching and pairs-up with a new mate, whereas in the Kentish plovers both parents rear the young until fledging after approximately 25 days.

Monitoring and marking of breeding adults and chicks has been carried out since 2006 and 2007 in Ceuta and Maio, respectively (see details in Székely et al. 2008). We carried out nest searching using a car and/or mobile hide. We captured nesting parents (using funnel traps) and chicks to ring them with a numbered metal ring, and adults additionally with an individual combination of color rings (see details in Székely et al. 2008). Previous intensive ringing efforts allowed us to individually identify between 70-80% and 80-90% of breeding adults in Ceuta and Maio, respectively.

Behavioral observations

In each site we searched for pairs with signs of active courtship behavior (e.g. copulating, flat running), building scrapes (shallow depressions in the soil where eggs are laid later on), and territory defending (e.g. fighting intruders away) using a car or mobile hide and binoculars. Male plovers usually defend a territory which then females adopt and defend. Although courting and copulatory behaviors can occasionally be seen in neutral grounds as feeding areas, they mostly take place within the territory (Cramp and Simmons 1985). When we found a pair in its presumed territory (non-feeding areas that individuals defend), we recorded their color ring combinations, location (UTM coordinates), distance from observer and time. Ten minutes after arrival (to allow for the habituation of the subjects to the observer), we recorded the following behavioral categories for each focal male and female by instantaneous sampling (Martin and Bateson 2009), every 20 sec during 30 min (90 records per observation): *courting with mate*, fighting with intruders, pecking at prey items or preening. Following descriptions of a 'Scrape-ceremony' and 'Mating-ceremony' by Cramp

and Simmons (1985), we classified the following behaviors, all of which are present in both sexes, under the *courting with mate* category: side-throwing, scraping, standing by the scrape, standing opposite a mate, flat running, cloaca showing and copulations. Both species observed in this study present similar sexual behavior and we detected no different postures or displays to the ones already described (details in Cramp and Simmons 1985).

We recorded one to four 30 minute observations per pair (mean ± se: 2.30 ± 0.22 complete observations per pair), each observation was made one to five days apart (2.81± 0.84 days apart, including two exceptional made 14 and 20 days apart, from re-nesting pairs) in the morning or afternoon. The proportion of time spent courting was consistent between morning and afternoon observations (paired Wilcoxon Signed-Rank test: Ceuta W = 1, $P > 0.20$, n = 4 pairs; Maio W = 3, $P > 0.50$, n = 5 pairs). All observations lasted 30 minutes and whenever focal subjects were hidden from our sight we recorded them as not seen. On average, in 8.7% of records individuals were unseen or covered and therefore no behavior was recorded. If individuals left the area and were out of sight for more than 5 minutes, the observation was stopped and deemed incomplete. We verified that scrapes found had no eggs to ensure that pairs had not initiated incubation yet and were observed in a similar reproductive stage as we were unable to know laying dates for all pairs. We observed a total of seven breeding pairs in Ceuta and 13 pairs in Maio. To maximize our sample size and avoid pseudoreplication, we used in the analysis only the first complete observation for pairs that were observed more than once in Ceuta (six pairs) and Maio (eight pairs). Of seven pairs observed in Ceuta, three had both adults color ringed, three only one adult color ringed and one pair had neither adult color ringed. In Maio, all pairs observed had both adults color ringed. We identified unringed birds in Ceuta using unique individual characteristics (e.g. plumage marks or limping) and their location, since snowy plovers are highly territorial (Warriner et al. 1986). All behavioral observations were recorded by the same observer (MCC-I).

Statistical analysis

To analyze the difference in the proportion of time spent courting between species we counted the total number of records under the *courting with mate* category and the total number of effective records (90 records per observation minus records where the focal subject was covered or not seen) across the first observation of each male and female. Using the total number of courtship records and the total number of effective records with no courtship, we analyzed the proportion of time spent courting within each species separately

for males and females using Generalized Linear Models (GLMs) with a logit link function and a quasibinomial error structure to account for overdispersion (Crawley 2003). Quasibinomial error structure is appropriate for overdispersion rates of up to ten (Crawley 2003); in our models overdispersion rates were 7.7 and 8.4 respectively in males' and females' model. However, we further tested the robustness of our results comparing courtship ratios of both species using a non-parametric Mood's median test which accounts for unequal variances (Kasuya 2001).

To investigate differences in the proportion of time spent courting by males and females (courtship bias) between species we used a linear model (LM) using the z-score of the difference between proportions of time spent courting by males and females as response variable. The main factor included in all models was species with two categories: snowy plover (polygamous) or Kentish plover (monogamous). We also included the date of observation (standardized) in our model to control and test for potential within-season variation in courtship, and the two-way interaction between the species and date of observation. Because of the small sample (n = 20 pairs), we restricted the models to two explanatory variables and one interaction to avoid overparameterization (Crawley 2003). From saturated models that included all explanatory variables and the two-way interaction of interest, non-significant interaction and terms were successively backwards eliminated starting with the largest p-value until minimal adequate models were reached (Crawley 2003). We report the significance of the increase in deviance resulting from model simplification (using F tests for GLMs and chi-square tests for LMs) as well as the coefficients for all variables kept in every final model. The variance inflation factor (VIF) (excluding interaction terms) was < 5 in all saturated models; hence, our results might not be biased by multi-collinearity. We checked each model for highly influential cases using residuals versus leverage plots and fitted models without points with leverage > 0.4 (Crawley 2003). Models including and excluding points with leverage > 0.4 yielded consistent results. We assessed the goodness of fit of saturated models using residual plots (Crawley 2003). All analyzes and figures were carried out using the 'base' package in R (R Development Core Team 2014, Version 3.1.0).

Results

Courtship behavior of males and females

Males and females in the polygamous snowy plover spent a significantly greater proportion of time courting than males and females in the monogamous Kentish plover (GLM: males difference between species: 25% , 17% – 26% [mean, 95% CI], $P < 0.01$, n = 20 males,

Table 1a, Fig. 1; GLM: females difference between species 14% , 8%–18%, $P < 0.001$, n = 20 females, Table 1b, Fig. 1). The difference between species in males and in females remained significant when using a non-parametric test (median test: males: $\chi^2_1 = 10.76$, $P < 0.01$, n = 20; females: $\chi^2_1 = 6.28$, $P = 0.02$, n = 20).

Table 1 Courtship behavior in two plover species: polygamous snowy plovers and monogamous Kentish plovers. Proportion of time spent courting in: a) males and b) females (Generalized Linear Models, GLM) and c) sex bias in courtship behavior (Linear Model, LM). Variables retained in the final models are shown in bold, n = 20 pairs. Statistics presented are from model comparisons using F-tests (GLM) or χ^2 tests (LM)

Models	**Model simplification**			
	ΔDev	**df**	**F**	***P***
a) Male courtship (% time)				
Species	211.76	1	23.45	**<0.001**
Date of observation	6.26	1	0.67	0.42
Date of observation * Species	9.80	1	1.06	0.32
b) Female courtship (% time)				
Species	153.12	1	18.01	**<0.001**
Date of observation	4.57	1	0.51	0.49
Date of observation * Species	0.05	1	0.01	0.94
c) Sex bias in courtship behaviour				
Species	3.72	1	**-**	**0.04**
Date of observation	1.25	1	-	0.22
Date of observation * Species	3.78	1	**-**	**0.01**

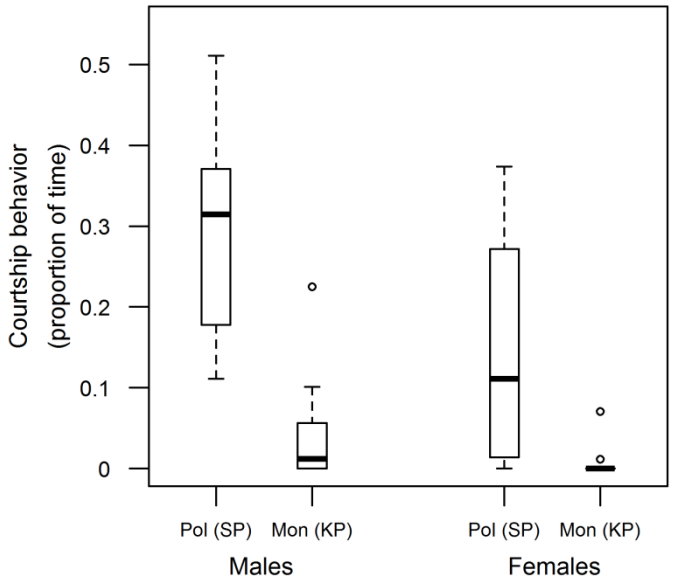

Fig. 1 Courtship behavior (proportion of time spent courting) by males and females in the polygamous (Pol) snowy plover population (SP, 7 males and 7 females) and in the monogamous (Mon) Kentish plover population (KP, 13 males, 13 females). Boxplot shows medians (bold lines), 25% and 75% quartiles (boxes). Whiskers show extreme data points within 1.5 times the interquartile range. Open circles show outliers lying 1.5 times outside the interquartile range.

Within-season variation

The significant interaction between date of observation and species indicated that courtship behavior had different temporal change between species (LM: difference between slopes of species: -0.97,-1.75 – -0.19, P = 0.03, n = 20 pairs, Table 1c, Fig. 2). In the polygamous snowy plover, females courted more than males early in the breeding season, whereas later on, males courted more than females (LM: 0.91, 0.27 – 1.55 [β, 95% CI], P = 0.01, n = 20 pairs, Table 1c, Fig. 2). However, in the monogamous Kentish plover, sex bias in courtship behavior was not related to the date of observation (Fig. 2).

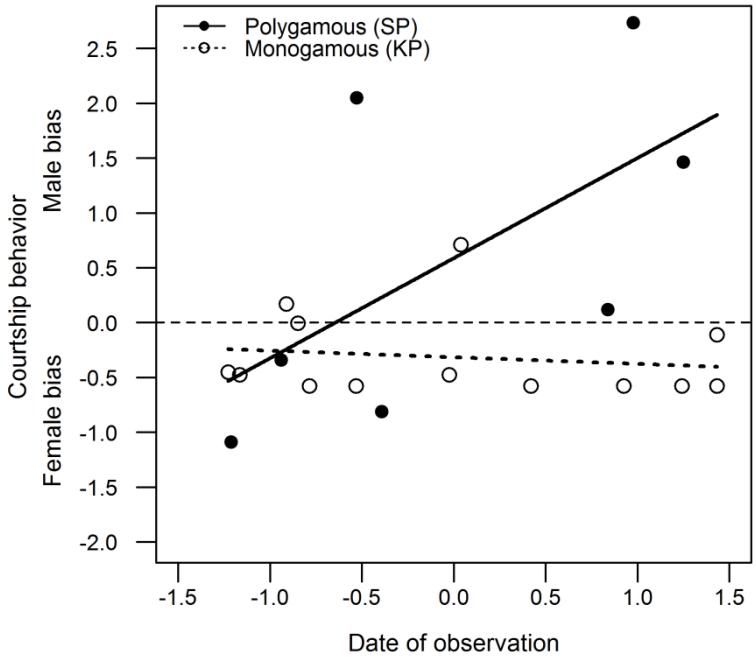

Fig. 2 Sex bias in courtship behavior (z-score of difference between males' and females' proportion of time courting) in relation to the date of observation in polygamous snowy plover population (SP, filled circles and continuous line) and in the monogamous Kentish plover population (KP, open circles and broken line), n = 20 pairs.

Discussion

Courtship behavior of males and females

We found that both males and females spent more time courting in the polygamous snowy plover than in the monogamous Kentish plover. This result supports the prediction that levels of mating competition and sexual selection are higher in polygamous species (Shuster and Wade 2003) as these show a higher degree of sexual dimorphism (e.g. Lindenfors and Tullberg 1998; Baker and Wilkinson 2001; Dunn et al. 2001; Ord et al. 2001; Thorén et al. 2006; Price and Whalen 2009) or more ornaments (Møller and Pomiankowski 1993) than monogamous species. This study adds the behavioral perspective to the evidence that mating competition and sexual selection is higher in polygamous than monogamous species.

The difference in the proportion of time spent courting between species could emerge in three ways. First, in a polygamous population, males and females may spend a long time competing to access mates and mate more than once; in this scenario males and females would have high courting rates to outperform others and breed. In monogamous populations,

males and females might be subject to strong mating competition upon their first reproductive event or after a divorce but not in successive events as re-mating with the same partner can reduce the costs associated to mate sampling and bond formation (Adkins-Regan and Tomaszycki 2007; Sánchez-Macouzet et al. 2014). In Maio over 90% of mates keep the same mate every year (O. Vincze et al. unpubl. data), so mates familiar with each other might not need to court as frequently as newly formed pairs.

Second, if courtship rate is an indicator of individual quality as suggested by many studies (e.g. Kotiaho 2002; Pariser et al. 2010), then individuals will increase their fitness by preferring mates with high courtship rates. In the polygamous population as individuals will breed more than once, mate sampling will allow them to compare between potential breeders, and therefore, mates with high courtship rates should have increased probabilities of being preferred. In the monogamous population mate sampling might happen upon the first reproduction or after a divorce, but as generally individuals keep the same mate in subsequent reproductions, frequency of mate sampling can be low and therefore they will rarely compare between mates with high/low courtship ratios. As emitting courtship displays might be energetically costly (Clark 2012), in a monogamous scenario were mates stay together, high courtship rates are unnecessary and minimum court rates could have a pair bonding and continuing mate evaluation purposes (Wachtmeister 2001).

Third, courtship behavior may differ between snowy plovers and Kentish plovers as a result of their intrinsic genetic differences. Although these species are genetically related, exhibit similar life histories, and were studied in comparable tropical localities, we cannot discard this potential explanation. A more robust comparison would involve several populations of the same species from ecologically equivalent sites but with different mating systems, which are often hard to find. The Saudi Arabian and United Arab Emirates Kentish plover populations pose an interesting alternative as they have contrasting mating systems and are both in desert regions (Kosztolányi et al. 2009; AlRashidi et al. 2011). Nonetheless, we deem unlikely that differences in courtship are explained just by the genetic differences between species. Indeed, genetic differences themselves might not give rise to differences in courtship behavior at least in fruit-flies: males of polygamous and experimentally selected monogamous lines held under controlled conditions show similar courtship rates (Hollis and Kawecki 2014). Differences in courtship are more likely given by social or environmental factors rather than only genetic differences.

A social factor that has proved to be related to the strong link between mating systems and competition for mates (Shuster 2009; Parra et al. 2014) is the proportion of males in the adult population (adult sex ratio, ASR) (Liker et al. 2013, 2014). While polyandry is related to more male-biased sex ratios, monogamy is related to unbiased sex ratios. Mating competition may increase if the number of one sex increases and the access to mates of the opposite sex is restricted. In line with this, a male-biased sex ratio in the polygamous snowy plover population in Ceuta (unpubl. data) may create high mating competition that leads to the high courtship rates observed. Contrastingly, if numbers of males and females in the population are similar, no strong mating competition is expected. Numbers of males and females in the monogamous Maio Kentish plover are largely the same showing a 1:1 ratio according to field surveys (ratio of males to females: 0.89 ± 0.06, 95% CI (0.76 to 1.03), t-test: t_{10}= -1.67, P = 0.12, n = 11 surveys, MCC-I and TS unpubl. data) and therefore no strong competition is expected leading to low courtship rates observed.

In contrast to theoretical predictions, a meta-analysis of experimentally manipulated mating competition (estimated as the operational sex ratio, OSR) found no relationship between courtship behavior and a change in OSR (Weir et al. 2011). The latter meta-analysis study used a number of fish and insect species with contrasting mating and care systems including a number of monogamous and polygamous species. However, if mating system might have an effect in how species respond to shifts in OSR, this was left unmentioned throughout the paper and the type of mating system shown by each species was not controlled for in the analyzes. It is currently not known whether monogamous species are responsive to changes in OSR at all or to what extent their courtship rates vary to those of polygamous ones. As our study suggests the mating system may be a key trait that must be considered in meta-analyzes of the kind.

In our study we could not control for further potential sources of variation in courtship behavior like laying date, male or female ornament variation, individuals breeding history, or age. However, in both sites we observed all the pairs encountered in non-feeding territories defended against intruders and being scraped, suggesting that pairs were establishing a nesting territory so presumably, the mate choice process had already occurred. We ensured that all pairs were in a similar reproductive stage by limiting our observations only to those where no eggs had been laid. Intriguingly, the number of pairs observed copulating and frequency of copulation during our study were very low in both species. Only in 42% of snowy plover pairs and in 23% of Kentish plover pairs 1-2 copulations were recorded in this study (data not shown). High frequencies of copulation in birds have been related to risk of

extra-pair paternity (Birkhead et al. 1987), so low frequencies of copulation observed in this study are consistent with the low rates of extra-pair paternity found in both species.

Within-season variation in courtship behavior

Sex bias in courtship behavior varied within the season in the polygamous snowy plover but not in the monogamous Kentish plover. In the polygamous population of snowy plovers in Ceuta, male courtship behavior (relative to female) intensified as the season progressed. We propose four non-mutually exclusive explanations for this result. First, early breeding snowy plover males may be in better condition than males breeding later on as is often the case in other bird populations (e.g. Dobson et al. 2008; O'Brien and Dawson 2013). Being of better quality or condition and potentially more attractive than later breeders, early breeding males might not need to court as much as poor quality late breeding males. For example, attractive male guppies courted less often than unattractive males, and this intensity was further modulated by environmental conditions such as ambient light (Reynolds 1993).

Second, the change in snowy plovers' courtship behavior might be responding to changes in OSR. Californian polygamous snowy plover populations have a male-biased adult sex ratio (Stenzel et al. 2011) and similarly Ceuta presents a male-biased sex ratio (unpubl. data); even though male snowy plovers provide more extensive care than females in the polygamous Ceuta population, OSR is still expected to become more male-biased because females will rapidly engage in breeding. An increasingly male-biased sex ratio with time of the season should lead to more intense mating competition and courtship behaviors in males, whereas for females mating competition should decrease. This matches the changes of courtship intensity we observed in the snowy plover in Ceuta (this study). On the other hand, in Maio males and females have comparable involvement in parental care and the OSR in this population might not be biased. Therefore, mating competition might be constant throughout the breeding season in this population and consequently no temporal change in the proportion of time spent courting rates was observed.

Third, a male bias in the snowy plover population means that late breeding males may have been mating for the first time whereas late breeding females were breeding for the second or third time (as female re-mating after brood desertion is common in this population). This male bias in addition to the potential decline in breeding success for late breeders documented in many bird species (reviewed in Verhulst and Nilsson 2008) could result in early breeding females accelerating their mate-choosing and courting phase compared to late ones. This could benefit females by maximizing both chick survival and their time

remaining for re-mating, resulting in early breeding males needing to court less than late ones.

The last alternative explanation to the seasonal pattern of courtship in snowy plovers from Ceuta is that of terminal investment. According to life history theory, if probability of future reproductions declines then an increase in current reproduction is expected (Clutton-Brock 1984). Late breeding snowy plovers might be in lower condition than early breeders and if their low condition reduces their probabilities of surviving until next year they might be willing to invest more in that breeding attempt. However, under this explanation it is unclear why this pattern was not observed in Maio's Kentish plovers, as the same prediction should hold under a monogamous mating system if probabilities of surviving from one season to the next one are similar in monogamous and polygamous populations. Experimental studies in species or populations with different mating systems will help disentangle the underlying causes of the temporal variation in courtship.

In conclusion, we found that closely related species of plovers with contrasting mating system differed in their time spent courting: the polygamous species spent more time courting than the monogamous one. However, the relationship between courtship behavior and mating system was more complex than initially thought given a within-season variation was found only in the polygamous species. Altogether our results suggest that courtship behavior may offer a simple and flexible tool to gauge the mating demand of males in relation to females. Despite moderate sample sizes, the species differences reported here were statistically significant, suggesting large effect sizes. For testing the generality of our observations, comparing courtship behavior across different mating systems in other populations or closely related species appears to be a fruitful avenue which may not require large sample sizes. Mores studies are needed to understand how sexual selection (and different estimates of its intensity) influences mating system evolution. We encourage future studies that combine behavioral estimates of sexual selection (e.g. aggression and courtship behavior) and compare these to other indices of sexual selection across species and populations with diverse mating systems.

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Ethical Standards: In this study we investigated two geographically widespread and non-endangered bird species in their natural habitats. The data collected for this study were based only on observations that did not require any capture or manipulation; data collection consisted on observations of pairs at a distance of 10 to 20 meters using a hide or a car, carefully avoiding the disturbance of the normal activities of birds. When using a car we drove only on marked tracks where cars usually drive in order to avoid additional disturbance of the natural habitat. As part of the annual monitoring, birds were caught using funnel traps during late incubation or after hatching of the clutch. Traps were left on the nest/clutch up to 25 minutes and were shaded to avoid egg exposure to heat, trapping was avoided at extreme heat. Birds were ringed and manipulated by well-trained people. All aspects of fieldwork were authorized by the national authorities in Cape Verde (Direcção Geral do Ambiente, DGA) and Mexico (Secretaría de Medio Ambiente y Recursos Naturales, SEMARNAT).

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4 Does social environment predict duration of female care in a polygamous bird?

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MCC-I: study conception and design, data extraction and estimations, statistical analysis, manuscript preparation
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Abstract

Parental care is a common social behaviour that aims to increase survival chances of the young. By care for the offspring, the parents trade-off the benefit of care against the benefits of terminating care and reproducing again. Social environment is hypothesised to facilitate early termination of care by allowing the deserter to find a new mate and breed again. Here we investigate whether two aspects of social environment (adult sex ratio, ASR, the proportion of adult males in the adult population; and/or operational sex ratio, OSR, the proportion of sexually active males in the sexually active population) are related to the duration of female care in the snowy plover *Charadrius nivosus,* a species in which females facultatively desert their brood and breed with additional partners. We predicted that duration of female care should be shorter when mating opportunities are high, i.e. at male-biased ASR and OSR. However, we found that with increasingly male-biased ASR (but not OSR) the duration of female care increased. Our work suggests that the relationships between ASR, OSR and parental care are complex since shifts in sex ratio may not only influence mating opportunities, but also the costs of re-mating or the risk of infanticide, and thus impact on both the benefits of care and the benefits of offspring desertion.

Keywords: adult sex ratio, operational sex ratio, parental care, social environment, mating system, breeding system, brood desertion

Introduction

Parental care is a taxonomically widespread behaviour that is expected to occur when a parent can enhance the development and survival of its offspring (Royle, Smiseth & Kölliker, 2012). A major aim in studies of parental care is to understand the factors that influence its duration and the extent to which it is provided by the male, the female or both parents (Clutton-Brock, 1991; Royle et al., 2012; Székely, Webb, Houston & McNamara, 1996). The form how parents care for their offspring and the amount of time and resources they invest on it depend on the balance between the benefits and costs of providing care. For example, parental care consumes time that could be devoted to self-maintenance, acquire additional mates, or carry out new breeding attempts (Royle et al., 2012; Smith, 1977; Székely et al., 1996; Trivers, 1972).

A key factor affecting parenting decisions is the social environment (Alonzo, 2010; Davies, 1992; Liker, Freckleton & Szekely, 2013; Székely, Webb & Cuthill, 2000; Székely et al., 1996). On the one hand, the proportion of sexually active males in the sexually active population (OSR, Emlen & Oring, 1977) may determine the number of potential new mates for members of each sex, and therefore, the amount of time and resources they allocate to care for their current offspring versus acquiring new mates. This expectation is supported by evidence from fish, birds and human hunter-gatherers, showing that when one sex is overrepresented in the sexually active pool of the population, the other sex tends to provide less care or desert the family to breed with additional partners (Keenleyside, 1983; Magrath & Komdeur, 2003; Marlowe, 1999; Pilastro, Biddau, Marin & Mingozzi, 2001; Poirier, Whittingham & Dunn, 2003). Therefore, if OSR is biased, it is expected that re-mating opportunities for the rare sex increase leading to parental desertion and forcing the remaining parent to provide care alone (Kokko & Jennions, 2012).

On the other hand, ASR (proportion of males in the adult population), may also influence parental care (Kokko & Jennions, 2008). Recent theoretical studies suggest that under male-biased ASR, males should give up multiple matings and focus more on parental care, whereas under female-biased ASR the opposite pattern is expected (Kokko & Jennions, 2008; Lessells, 2012; Székely, Weissing & Komdeur, 2014). This prediction has been supported by observations in fish (Balshine-Earn & Earn, 1998; Keenleyside, 1983), bird (Burley & Calkins, 1999; Liker et al., 2013) and most polygynous mammal species (Clutton-Brock, 1991) where parental care is provided by the most abundant sex.

Additionally, ASR may influence the costs and benefits of care for offspring. For example, females may remain with their current family instead of looking for new mating opportunities if male harassment or aggression toward females increases under male-biased ASR, and this threatens their survival and/or survival of their developing offspring (e.g., eggs, foetuses, chicks or pups) (Anderson, Porter & Ferree, 2004; Hailey & Willemsen, 2000; Le Galliard, Fitze, Ferrière & Clobert, 2005; Taylor, Leonard & Boness, 2001). Consequently, impacts of ASR on individual parental decisions can be expected.

The intricate relationships between ASR, OSR and parental care have been addressed by theoretical studies (Houston, Székely & McNamara, 2013; Kokko & Jennions, 2008; McNamara, Székely, Webb & Houston, 2000; Webb, Székely, Houston & McNamara, 2002), but empirical evidence of how both ASR and OSR impact on parental cared is still limited. ASR is the base from which OSR is derived: "*the OSR is the ASR corrected for the difference in care by each sex that determines their 'time out' of the mating pool*" (Kokko & Jennions, 2012). Therefore, demographic precursors of ASR, such as sex differences in survival associated with differential costs of reproduction (Székely, Weissing, et al., 2014), will necessary influence variation in OSR, and ultimately, sex-specific re-mating opportunities and patterns of parental care in a population. Parental care may also influence directly OSR because the amount of time individuals devote to care for their offspring will determine their 'time out' periods from the mating pool. Similarly, differential investment in parenting behaviour and mating competition may entail differential mortality costs for males and females (Liker & Székely, 2005) which in turn may lead to biased ASR (Székely, Liker, Freckleton, Fichtel & Kappeler, 2014), and further biases in OSR. Biological implications of ASR and OSR, however, must be investigated separately (Jennions & Kokko, 2010) because these ratios might not be always correlated (Székely, Weissing, et al., 2014; Carmona-Isunza et al. in review) and may reflect different processes since their underlying causes are dissimilar (Székely, Weissing, et al., 2014).

Here we quantify both ASR and OSR in a wild population and investigate whether both or one of these ratios predicts duration of female care in a small shorebird, the snowy plover *Charadrius nivosus*. Snowy plovers are an excellent ecological model system to investigate how social environment affects parental care for two reasons. First snowy plovers have flexible parental care since one parent (usually the female) abandons the brood at variable chick age so that some broods have no biparental care, whereas in other broods both parents stay until the

chicks fledge at age 25 - 31 days (Warriner, Warriner, Page & Stenzel, 1986). Deserting snowy plover females are often polygamous, i.e., re-nest with new mates shortly after deserting their former mate and brood (Warriner et al., 1986). Second, preliminary data suggest that breeding activity and both ASR and OSR are variable throughout the breeding season (April-June, Carmona-Isunza, Küpper, Serrano-Meneses & Székely, 2015). Using data from a marked population spanning three years (2009-2011), we examined natural variation in the duration of female care in relation to variations in both ASR and OSR. Specifically, we expected that female plovers would have short parental care if more males are in the population (i.e., male-biased ASR) and/or if more males are available for mating (i.e., male-biased OSR).

Methods

Population monitoring and sampling

Fieldwork was carried out during the breeding season (April-July) in Bahía de Ceuta, Sinaloa, Mexico (23º54 N, 106º57 W) where 60 – 200 snowy plovers breed in a ~150 ha salt marsh (Cruz-Lopez et al., in prep.). Ringing and population monitoring started in 2006, and the data for the current study were collected in 2009, 2010 and 2011. Fieldwork covered 93 days in 2009, 71 days in 2010 and 81 days in 2011. We searched for nests and continuously monitored egg survival until hatching, when chicks were ringed. We trapped unringed adults during incubation using a funnel trap and ringed them with an individual metal ring and colour ring combination. From 2006 until 2011, around 500 adult snowy plovers have been marked using metal rings and colour ring combinations. For families that were encountered after the chicks had left the nest, chick age and hatching date were estimated using chick's tarsus length assuming linear growth (see details in Székely & Cuthill, 1999). Upon hatching, we monitored broods every 2-4 days to record chick survival and the sex of the attending parent(s). If only one parent was present when a brood was found, the brood was observed for 15 min or until the missing parent was seen.

We re-sighted previously banded adults throughout the breeding season using a spotting scope or binoculars, noting date, time, colour ring combination, sex based on plumage characteristics (in snowy plovers similarly to Kentish plovers males have darker ornaments than females, Argüelles-Ticó et al., 2016) and/or behaviour (i.e., feeding, courting, preening) and location. We also carried out population surveys every two weeks during the breeding season, from 2009 to2011. In surveys, observers stopped every 100-200 m moving inside a mobile hide through

the entire study area in order to record colour ringed plovers identities, sex, date, time and location.

To estimate ASR and OSR we used a methodology developed for another study (Carmona-Isunza et al. in review), we only provide here a brief overview of the methodology followed. To estimate ASR and OSR we used data from 111 adults (males: 46, 38, 24; females: 27, 32, 21 in 2009, 2010 and 2011, respectively; individuals may have bred throughout the three years) that had been marked with individual colour id combinations before the focal year. For every focal adult, we estimated their reproductive events between arrival and departure to and from the breeding area. We defined the day when a colour ringed plover was first seen as “arrival date”, and the day when it was last seen as “departure date” based on all re-sighting and nesting data available.

An adult was considered unavailable for mating (i.e., “time-out”) ten days before completing the clutch and until the last day of parental care. Ten days is the usual time period between establishing the pair bond and producing a three egg clutch (modal clutch size). Clutch completion date was estimated for clutches found after egg laying had finished by floating eggs in water (eggs lose water during incubation and their density decreases gradually) when the nest failed or from observed hatching dates assuming an incubation period of 25 days (Székely, Kosztolányi & Küpper, 2008). The last day of parental care was taken as the date when (i) eggs were lost, (ii) chicks died or (iii) fledged, or the date of desertion (see *Duration of female care*). The remaining days between arrival and departure when the adult was not involved in a reproductive event were considered as available to breed (“time-in”). We used arrival, departure dates and “time-in” periods to compute daily ASR and OSR estimates for the three years, separately.

Estimating ASR

We used the intervals of time between arrival and departure of each individual to estimate ASR. Using the sample of 111 breeders described above, we estimated ASR counting the number of males and females from our sample that were present in the population (based on the arrival and departure dates) on any given day of the breeding season. ASR was the number of males present divided by the total number of males and females present multiplied by 100 to reflect a percentage.

Estimating OSR

Using the same sample of 111 breeders, we counted how many males and females were present and available to breed on any given day of the breeding season according to their arrival, departure dates and to their "time-in" periods. OSR was the number of males present and available to breed divided by the total number of males and females that were present and available to breed multiplied by 100.

Duration of female care

Duration of female care was the number of days between the hatching date and the date when the female deserted the brood; if female was attending the brood (censored observations, details below), we estimated duration of female care as the number of days between the hatching date and the date when the chicks died, fledged, brood was not seen again or fieldwork was terminated. A female was considered to have deserted its brood only if it was not seen for at least two consecutive brood checks while the chicks were still alive (mean days between brood checks: 1.97 ± 0.057, n = 1,292 observations of 154 broods). The date of desertion was the midpoint between the last day the female was seen caring for its brood and the first day it was recorded absent.

Statistical analyses

Using broods that were seen at least three consecutive times during a breeding season, we gathered a sample of 109 nests belonging to 69 females that bred in 2009, 2010 and/or 2011. Sex of females considered in this analysis was confirmed with previous molecularly sex-typing (methods described in: Dos Remedios, Székely, Küpper, Lee & Kosztolányi, 2015). We used Cox proportional hazards regression models (referred as Cox model, David Machin, 2006) to investigate whether ASR and/or OSR influenced duration of female brood care. Since we had 23 females that bred up to four times in the three years, we used the female's identity in a clustered Cox model (David Machin, 2006) to account for the non-independence of the data. A terminal event was considered to occur when the female deserted her brood. Censored observations were those in which females stayed with their brood either until chicks died, fledged, brood was not seen again or fieldwork ended in a given year. The duration of female care in our sample was not influenced by experimental manipulations previously carried out in this population (cross-foster experiment; 59 experimental vs 65 non-experimental broods; Kruskal-Wallis rank test: $\chi^2 < 0.10$, df = 1, $p = 0.75$). All desertions took place during an interval

of 26 days, i.e. between the 29th and 55th day of breeding. Goodness of fit and Cox model assumptions of proportional hazards over time were checked using a set of different residual plots for full models including ASR or OSR, all covariates and interactions of interest (Grambsch & Therneau, 1994; John Fox & Weisberg, 2002).

We used Akaike's information criterion adjusted for small samples (AIC_c) and weights (ω_i) to rank candidate models (Burnham, Anderson & Huyvaert, 2011) to test whether ASR explained more variation in the duration of female care than OSR. We fitted separate models for ASR and OSR to avoid collinearity given that they were correlated in our sample (Spearman rank correlation: r = 0.53, $p < 0.001$). Females often re-mate after deserting their broods (Székely, Cuthill & Kis, 1999; Warriner et al., 1986), and they probably assess their re-mating opportunities some days before desertion occurs. In order to account for this potential lag, we included mean values of ASR or OSR within five days prior to the end of females' care in our models. ASR and OSR refer to mean values within this five day period unless stated otherwise. As covariates we included year (a factor with three levels) and the standardized laying date in models in order to account for interannual and seasonal variation in parental care duration associated with unmeasured environmental conditions (e.g., food availability or resource competition). We also included the mean number of adults within five days prior to the end of female's care (denominator of the ASR estimation) as a covariate in order to account for population density in the breeding area, since density may influence parental care (Owens, 2002). We created a set of candidate models with all possible combinations of covariates and/or ASR or OSR. We only tested the interaction density x ASR or density x OSR. The model with the lowest AICc score was considered the model that best fitted the data. Models that did not differ in more than two units in their AICc scores ($\Delta AICc < 2$) were considered statistically equivalent (Johnson & Omland, 2004). We used unconditional model averaging (averaged over all models in the confidence set) using models within the 90% confidence set (Burnham & Anderson, 2002) to estimate coefficients of parameters and their confidence intervals in the best AIC model (Buckland, Burnham & Augustin, 1997; Symonds & Moussalli, 2011).

To test the sensitivity of our results to the estimation of ASR and OSR in the five-day period previous to the end of female care, we replaced this estimation with the value of ASR and OSR for the last day of care (date of desertion or date when chicks died or fledged) and the mean values of these ratios in a ten-day period previous to the end of female care. Mean values of

ASR within five days and within ten days prior to desertion, and ASR values estimated for the date of desertion were statistically different from each other (Wilcoxon paired tests, all *p* values < 0.02 after Bonferroni correction). Therefore, after finding the best model from the set of models described above (see "Results"), we used AICc scores to compare a set of three competing versions of that model differing in the ASR estimates used: mean values of ASR within five days, within ten days prior to desertion, and ASR values estimated for the date of desertion. Mean values of OSR within five days and within ten days prior to desertion, and OSR values estimated for the date of desertion were not statistically different from each other (Wilcoxon paired tests, $p > 0.28$ after Bonferroni correction), and therefore, comparisons of the predictive power of these three different OSR estimates were unnecessary.

All analyses and plots were generated with R (R Development Core Team 2014, Version 3.2.5 using libraries: 'base', 'survival' and 'AICcmodavg'). Unless otherwise stated, we provide mean values along with their standard errors.

Ethical note

In this study we investigated a geographically wide-spread and non-endangered bird species in its natural habitat. As part of the annual monitoring we used standardized methodologies. During late incubation or after hatching of the clutch, adults were caught using funnel traps. Traps were left on the nest/clutch for a maximum of 25 minutes and shaded to avoid egg/chick exposure to heat; we avoided trapping at extreme heat. Birds and chicks were ringed and manipulated by well-trained people. Fieldwork was authorized by the national authority in Mexico (Secretaría de Medio Ambiente y Recursos Naturales, SEMARNAT; permit number: SGPA/DGVS/03076/13).

Results

Duration of female care and sex ratios

In 80 of the 109 broods studied (73%), female desertion was recorded on average 4.4 ± 0.60 days (range: 0-23 days) after chicks hatched. In four broods (3.6%), females care until chicks fledged (25 days after hatching), and in the remaining 25 broods females were seen with their chicks at the last brood observation (total of 29 censored observations).

ASR was a better predictor of female care than OSR: the model with strongest support (w = 0.57) indicated that the duration of female care was related to ASR (Table 1), whereas models

including OSR had substantially weaker support in the data (w = 0.04, Table 1). Contrary to our expectation, male-biased ASR was associated with longer female care; when controlling for density, year and laying date, duration of female care increased roughly 13% with every percent increase in ASR (ASR hazard ratio [95% CI] = 0.87 [0.74-0.96], Figure 1).

Sensitivity analysis

To test the sensitivity of our analysis we re-ran only the best-supported model replacing the five-day interval mean estimate of ASR and density with mean values for the last day of care and for the ten-day period previous to the end of female care. From these three models, the model using estimates for the last day of care was better supported by the data (AICc: 554.8, w = 0.99), and the hazard ratios for ASR (0.89 [0.81-0.99]) were of similar magnitude and direction as those from the model with five-day interval values (reported above). The models using mean values of ASR and density within five and ten days before desertion had weaker support in the data (AIC: 563.5, 570.1, ΔAICc > 8, $w < 0.02$).

Table 1. Model selection results for Cox regression models analysing the influence of adult sex ratio (ASR) and operational sex ratio (OSR) on duration of female brood care in snowy plovers (*Charadrius nivosus*). Models are listed according to their fit from best to worst, only the ten highest ranked models are shown (n = 109 broods belonging to 69 females).

Model description	*K*	AICc	ΔAICc	ω_i
y~dens + year + LD + ASR+ cluster(id)	5	563.46	0.00	0.57
y~dens * ASR + LD + year + cluster(id)	6	565.59	2.14	0.20
y~dens + LD + year + cluster(id)	4	566.87	3.42	0.10
y~dens * OSR + LD + year + cluster(id)	6	568.80	5.35	0.04
y~dens + year + LD + OSR + cluster(id)	5	568.95	5.49	0.04
y~dens + year + ASR + cluster(id)	4	569.33	5.87	0.03
y~dens * ASR + year + cluster(id)	5	571.43	7.98	0.01
y~dens + year + cluster(id)	3	572.12	8.67	0.01
y~dens + year + OSR + cluster(id)	4	574.15	10.69	0.00
y~dens * OSR + year + cluster(id)	5	575.04	11.59	0.00

K, number of parameters; AICc, Akaike Information Criterion corrected for small sample sizes; ΔAICc, difference between current model and the previous best-fitting model in their AICc scores; ω_i, relative support for every fitted model**.** LD: standardized laying date, year: factor with three levels; * indicates main terms plus their interaction. The model in bold corresponds to the one with best fit according to the ΔAICc ≤ 2. Coefficient estimation of parameters in the best model was done using model averaging for the first four models adding a ω_i = 0.91.

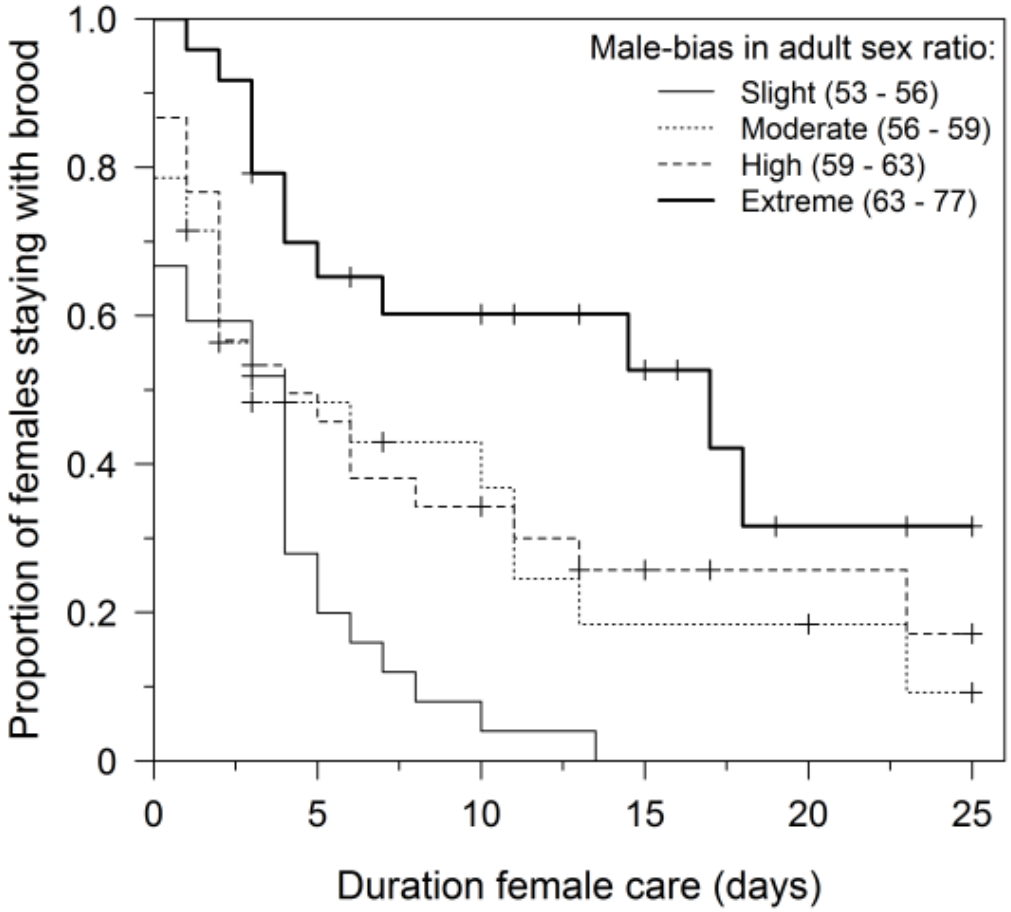

Figure 1. Predicted Kaplan-Meier curves for the duration of female care under different biases of adult sex ratio estimated in a five-day interval prior to females terminating their care. The categories are defined by adult sex ratio's quartiles and are shown as percentages.

Discussion

This study provided two key results: ASR was a better predictor of female care than OSR, and females stayed with their broods for a longer time when ASR was more male-biased.

We are unaware of any other study that has evaluated natural temporal variation in both ASR and OSR to compare their predictive power over parental care, and more specifically, over female care decisions. ASR was a better predictor of female care probably because it is less variable over time than OSR in the snowy plover population of Ceuta (Carmona-Isunza et al. in review). Less variability could mean that ASR provides more accurate and longer lasting information to females than OSR about the cues they use to assess the costs and benefits of staying with their brood versus abandoning their current offspring and mating with additional partners. Empirical examinations of the impacts of sex ratios on parental care have focused exclusively on male participation in care (incubation and feeding of young), or male brood desertion (Keenleyside, 1983; Magrath & Elgar, 1997; Marlowe, 1999; Poirier et al., 2003; Westneat, 1988; but see Pilastro et al., 2001), leaving open the question of how sex ratios influence parental behaviour in females. Moreover, the aforementioned studies so far have either set different experimental sex ratio conditions in the lab or counted the number of unmated/fertile females in a specific radius or the number of available mates per year (Keenleyside, 1983; Magrath & Elgar, 1997; Marlowe, 1999; Pilastro et al., 2001; Poirier et al., 2003; Westneat, 1988), without making a clear distinction between ASR and OSR. Although more empirical studies in different taxa that consider the dynamic nature of ASR and OSR are necessary to confirm the broadness of our findings, we suggest that in general, ASR is a better predictor of parental care than OSR because its demographic origin makes it more stable and reliable through time than OSR, which has its origin in ASR plus the behavioural responses of individuals (Kokko & Jennions, 2008; Székely, Weissing, et al., 2014).

Mating opportunities have been appointed as an important factor in the evolution of parental care in theoretical models (Clutton-Brock, 1991; Smith, 1977; Székely et al., 2000; Székely et al., 1996). Empirical studies have generally considered the number of available mates as a proxie of OSR and have assumed this is an indicator of mating opportunities (e.g. Balshine-Earn & Earn, 1998; Breitwisch, 1989). Numerous studies in birds, fish and humans that relate parental care to OSR have found that males or females will provide less care when more mates are available (Keenleyside, 1983; Magrath & Elgar, 1997; Marlowe, 1999; Pilastro et al., 2001;

Westneat, 1988). Based on this generalised assumption, we expected that ASR, similarly to OSR, could signal mating opportunities and females would respond to this by reducing the duration of care in order to re-mate and produce additional offspring, which ultimately may increase their fitness. Contrary to the expectation, we found that under more male-biased ASR females stayed with their broods for longer. We suggest two alternative explanations to this unexpected result.

First, by staying with their broods, female snowy plovers could avoid or reduce potential costs associated with increased biases in ASR towards males. Male harassment may increase under male-biased ASR, and this increase may impose survival costs for females or put at risk the survival of their offspring, as it has been reported in lizards, turtles and birds (Anderson et al., 2004; Hailey & Willemsen, 2000; Le Galliard et al., 2005; Taylor et al., 2001). Fights between adults in which chicks may be injured or killed occur occasionally in the congeneric Kentish plover (Kosztolányi, Székely, Cuthill, Yilmaz & Berberoğlu, 2006), and more male-biased ASR may imply that an increased number of single or parenting males are defending territories or chicks. An increase in chick mortality due to a higher frequency of territorial fights between adults may shift the re-mating cost/benefit for females, so that they could opt to stay with their broods for longer until this risk diminishes and increase chick survival. In fact, chick survival estimates suggest a higher survival when mean annual ASR is more male-biased when presumably females stay for longer with their broods (Carmona-Isunza et al in review: ASR median = 0.64, 0.56, 0.60, n = 119, 131, 115 days; ; in 2009, 2010 and 2011 respectively; Crúz-López, Eberhart-Phillips et al. unpublished data: chick survival: 0.34 ± 0.05, n = 100 chicks, 0.05 ± 0.03, n = 135 chicks, 0.52 ± 0.06, n = 83 chicks; in 2009, 2010 and 2011 respectively).

Therefore, male-biased ASR might not exclusively signal additional mating opportunities to females as we had assumed. Whilst there is some evidence that suggests that female care may decrease as the number of available mates increases (Pilastro et al., 2001), it is possible that sex ratios provide mixed cues to already mated females. Male-biased sex ratios may signal mating opportunities to unmated females, increased risk of harassment and chick mortality to breeding females, or bad conditions if only a few females are willing to breed. Moreover, ASR (and/or OSR) could provide different cues to females depending on the breeding system and on the role they play in reproduction. Previous studies have focused on monogamous species with biparental care or species where mostly -or only- the female cares for the offspring

(Keenleyside, 1983; Magrath & Elgar, 1997; Marlowe, 1999; Poirier et al., 2003; Westneat, 1988; but see Pilastro et al., 2001), where the balance between benefits and costs of reducing parental care and mating with additional partners may be different in comparison with a polygamous species with sex-role reversal like snowy plovers.

Second, a delayed desertion at highly male-biased ASR could be a consequence of females optimizing their care. Offspring survival is reduced when females of the congeneric Kentish plover desert their broods (Székely & Williams, 1995), therefore females maximise their reproductive success by staying longer with their broods. When ASR is more male-biased, females could be sure that they will find a new male whenever the desertion takes place and therefore can afford to stay a few more days with the chick. However, if ASR is less male-biased, females will have to compete with other females to have access to another male and may therefore desert earlier to have higher chances of re-mating.

To our knowledge, this is the first study that relates sex ratio variation to female parental care in a polygamous species. Further studies in taxa with diverse breeding systems examining individual responses to sex ratio variation during different stages of breeding may provide valuable insights into the nature of these complex associations. It is important that future analyses test the impact of both ASR and OSR because it is unknown which is more influential in determining breeding behaviours and these ratios may provide different cues to unmated and already mated individuals.

Although behavioural responses often lag behind the cues to which they react (DeWitt, Sih & Wilson, 1998), and this may be explained by the need of assessing several cues when the environment is prone to change, we found that ASR did not have a lagged impact on the duration of female care. The impact of ASR on female care duration was similar when including a mean ASR from five and ten days prior to desertion, but ASR estimated for the day of desertion had stronger support in the data. This result suggests that females use ASR as a cue to assess the costs and benefits of staying with their broods close before desertion takes place. Notably, the impact of ASR on female care duration did not differ between years (i.e., the interaction between ASR and year of observation was not supported by the data) suggesting that females respond in a consistent way to ASR variations.

In conclusion, this study shows that the relationship between sex ratios and parental care is more complex than previously thought as bias in sex ratios may entail both benefits and costs for the less abundant sex and may provide mixed signals to already mated individuals. ASR may be a better predictor of parental care duration than OSR. We highlight the need to test the overlooked assumptions lying behind sex ratio theory to fully understand the cues and signals sex ratios may pose for individuals across different breeding systems.

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5 Breeding schedule of males and females in monogamous and polygamous plover populations

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Manuscript

This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.

Author contributions

MCC-I: study conception and design, fieldwork, data extraction and estimations, statistical analysis, manuscript preparation
LJE-P: fieldwork, data collection, data preparation, molecular analyses, manuscript improvement
CK: fieldwork, data collection, study design, manuscript improvement
MC-L: fieldwork and data collection
SZ: fieldwork and data collection
JH: fieldwork support, manuscript improvement, critical review
OK: fieldwork support
TS: study conception and design, fieldwork, data collection, statistical advice, manuscript preparation

Abstract

The time of arrival and duration of breeding (breeding schedule) are essential elements of both individual fitness and population dynamics. However, it is unknown to what extent these elements may vary in populations with different breeding systems. We therefore compared six populations comprising five related plover species that exhibit different breeding systems from monogamy with biparental care to polygamy with uniparental care. We hypothesized that sex-specific breeding schedules are reflected in local breeding system and sex-specific mating opportunities and predicted that monogamous populations would exhibit equal breeding schedules and polygamous populations would exhibit different breeding schedules for males and females. We found modest support for our predictions as only a single polygamous population revealed a significant difference between male and female breeding schedules, as males arrived earlier and had longer breeding schedules than females. We discuss the potential drivers of differences between our study populations and suggest that the extreme male-biased adult sex ratio seen only in the polygamous population that supported our predictions could be responsible for the sex-specific differences. We also point to the ecological and demographic implications that breeding schedules may have on the adult sex ratio and operational sex ratio.

Keywords: arrival time, protandry, duration of breeding, sex ratios, adult sex ratio, breeding systems, sex-roles, parental care, migration, plover

Introduction

On the breeding grounds, sex-specific selection pressures can influence arrival times and duration of stay for breeding individuals, two key components of individual fitness. Protandry (i.e. the earlier arrival of males to breeding grounds than females) is widespread across several taxonomic groups (e.g., arthropods, fish, reptiles, amphibians, birds and mammals, reviewed in Morbey and Ydenberg 2001). Protandry may arise from two mutually non-exclusive pathways. Firstly, males may attempt to maximize mating opportunities by arriving early to intercept females or occupy the highest-quality territories (Morbey and Ydenberg 2001; Kokko et al. 2006). This has been shown to increase mating success in males (Møller et al. 2009), but may depend on age (Cooper et al. 2009). Alternatively, females may delay their arrival to minimize the time spent waiting for males or to enhance food reserves on migratory stop-over sites (Morbey and Ydenberg 2001; Kokko et al. 2006). However, for protandry to evolve there must be a reproductive benefit for early arriving individuals given that survival costs from arriving early are likely (Kokko et al. 2006; Morbey et al. 2012).

Although protandry is widespread in migratory avian species (e.g. Spina et al. 1994; Rubolini et al. 2004), it is unknown if protandry occurs in resident species or in partial-residents as some or all individuals might not stay in the breeding grounds all year round. Protandry is expected to reflect the intensity of local sex-specific mate competition, with protandrous populations exhibiting a male-biased adult sex ratio (ASR, i.e. proportion of males in the adult population) and female-biased populations exhibiting protogyny (i.e. females arriving earlier than males; Kokko et al. 2006). While sex-specific arrival times have been explored under a theoretical framework, empirical evidence is lacking.

Another temporal component of breeding is the time of termination, which may also differ between males and females due to sex-specific parental roles. For example, if females are obliged to provide extended care of offspring (Székely et al. 1996), males are emancipated and can leave the breeding grounds to prepare for migration. Due to these sex differences in arrival and/or departure dates, the total breeding duration may differ between males and females. Collectively, these three temporal components make up a “breeding schedule”. Sex-specific differences in the arrival date, the number of breeding events, the amount of time spent searching for mates, and providing parental care are key determinants of the breeding schedules of males and females.

Sex-specific breeding schedules are predicted to be related to the prevalent breeding system of a population. Breeding systems are closely tied to local ASR biases, sex-specific mating opportunities and sexual selection pressures (Møller and Pomiankowski 1993; Liker et al. 2013; Liker et al. 2014; Parra et al. 2014; Carmona-Isunza et al. 2015), which may

favour or disfavour protandry or protogyny. Moreover, the breeding schedules of males and females may be comparable in monogamous populations with biparental care, whereas polygamous populations with uniparental care may exhibit longer breeding schedules for the sex providing most parental care or taking longer to secure a mate.

In this study we collected and analysed highly detailed individual-based breeding data from six populations comprising five closely related species of *Charadrius* plovers (Dos Remedios et al. 2015a) to test the hypothesis that sex-specific breeding schedules are reflected in local breeding system and sex-specific mating opportunities. We predicted that monogamous populations would exhibit equal breeding schedules and polygamous populations would have different breeding schedules for males and females. Plovers are excellent model organisms to test these predictions given their variable breeding systems which range from strict monogamy with biparental care to polygamous with male-only or female-only care (Lessells 1984; Székely and Lessells 1993; Székely and Williams 1995; Amat et al. 1999; Thomas et al. 2007). These species also exhibit appreciable inter- and intra-specific variation in ASR ranging from female-biased to highly male-biased (Carmona-Isunza et al. in review; Eberhart-Phillips et al. in prep).

Methods

Field work

We studied five closely related plover species constituting six populations from four localities (Table 1). Breeding areas in all of the locations consisted of saltmarshes surrounded by coastal dunes (Maio, Cape Verde), mangroves (Ceuta, Mexico), or dry upland habitats (Tuzla, Turkey; Andavadoaka, Madagascar). Plovers are small ground nesting insectivorous shorebirds (body mass 35-60 g) that have inter- and intraspecific variation in breeding system (Table 1). In socially monogamous populations, both parents provide care of offspring and pair-bonds may last over several breeding seasons. Polygamous populations have uniparental care and exhibit either sequential polyandry (i.e., snowy plover, Kentish plover in Tuzla; Table 1) or a mixture of sequential polyandry and polygyny (Kittlitz's plover). We classified the breeding system of each population based general patterns observed, however it should be noted that in all populations there is some degree of variation and occasional cases of polygamy or monogamy, uniparental care or biparental care.

Our field work followed a standardized protocol to find nests, estimate laying dates and hatching dates, and to capture and uniquely mark adults and chicks (see detailed methodologies in Székely et al. 2008) during local breeding seasons (months shown in

Table 1). Captured adults were measured (tarsus, ulna and beak), weighed, and ringed with an alpha-numeric metal ring and a unique colour combination, thus allowing us to identify individuals by non-invasive resightings. Newly discovered nests were visited every day until the clutch completion or, if clutch was complete upon finding, we visited every three days to monitor nest survival. When chicks hatched, broods were revisited every other day to assess chick survival and determine the identity of tending parent(s). When unidentified broods were found, chicks were captured, and the parents were identified or trapped. In these cases, hatch dates were estimated by measuring the tarsus and using population specific growth curves (Dos Remedios et al. 2015b), except in Andavadoaka where a general growth curve was used (Székely et al. 2008). During all captures, we sampled 25–50 µL of blood from the meta-tarsal vein of chicks and the brachial vein of adults for molecular sexing (see details below). Throughout the breeding season of each population we opportunistically recorded re-sightings of colour-ringed individuals during our daily field work.

In Ceuta and Maio, we carried out additional surveys consistently every two weeks or every week respectively throughout the breeding season of some years (Ceuta: 2009-2011, Maio: 2013-2015). During these surveys, either the whole area (Ceuta) or a pre-defined transect (Maio) was covered consistently using a hide (Ceuta) or a car (Maio) and we recorded the field sex and colour combinations of all individuals seen in a ~100 m radius using binoculars and a spotting scope.

Estimating arrival dates and breeding schedules

To estimate individual arrival dates and breeding schedules, we focused on unique colour marked individuals that were ringed in the season prior to the focal breeding season. This method improved the precision of the arrival date estimate since individuals newly marked half-way through the season were excluded. Our sample only included individual cases for which all variables were known (see *Statistical analyses* below).

We estimated the arrival date of individuals using information from re-sightings, surveys (where available), capture dates and nesting information, and it corresponded to the earliest date when an individual was seen or was presumably present according to the laying date using egg floatation tables and included a 10 day pre-laying period that accounted for pair-bonding. This 10-day period represents the midpoint of the 8-11 day average that it takes re-nesting females to establish a new partner and nest in Ceuta (Carmona-Isunza et.al unpublished data). The departure date was determined from the latest date an individual was seen during re-sightings or was presumably present in the breeding site according nesting or brooding of their last reproductive event in a given breeding season. We defined the length of breeding schedule as the time (in days) spent at the breeding grounds each

breeding season, and was estimated as the interval between the arrival date and the departure date of an individual.

Sexes were identified based on dimorphic plumage as males have darker head and breast bands than females in some plover species (Argüelles-Ticó et al. 2015) and confirmed by molecular sexing (details in Dos Remedios et al. 2015b) on most cases (plumage sexed: 211 individuals, molecular sexed: 184 individuals). The two methods were highly concordant, with 98.4% sexing accuracy being obtained in populations with both plumage based and molecular based sex determination (n=184 individuals). Sex identification based on dimorphic plumage was not possible in the three sexually monomorphic species from Madagascar (Table 1) where only molecular sexing was done. In Tuzla (Table 1), no molecular sexing was possible so only the plumage based sexing was used and this was highly reliable in individuals with multiple captures (100% sexing accuracy, n = 105 captures of 50 individuals). We excluded 15 individuals from our sample whose molecular sex was identical to that of their partners.

Statistical analyses

For all subsequent analyses we standardized arrival date and length of breeding schedule for each year and population (i.e. subtracting each observation from the mean and dividing by the standard deviation), enabling us to compare across years and populations. To better fit a normal distribution, we further transformed the standardized arrival date with a square root and the addition of a constant thus forcing all data to be positive and non-zero. Using these data, we carried out separate analyses for: (i) arrival date and (ii) breeding schedule length.

We carried out a multi-population analysis to understand whether arrival date and breeding schedule lengths are sex-specific and if these trends vary between breeding systems. We used a linear mixed model (LMM; fitted with maximum likelihood) with individual identity nested within population as a random factor. In these models we included sex and breeding system (factor with two levels: monogamous and polygamous) as fixed effects. Covariates included the total number of nests in the focal breeding season (total nests herein) to control for the potential earlier arrival or longer stay of individuals breeding more than once in the same year, and to control for individual quality: relative body size (i.e. mean tarsus length for all adult captures previous to the focal breeding season, standardized by population) and minimum age (i.e. number of years since first ringing). Two-way interactions of interest included were: breeding system * sex and sex * minimum age.

Table 1. Details of polygamous (P) and monogamous (M) species and populations studied. Uniparental (U) and/or biparental (B) care can be present in these plover species.

Breeding System	Species	Locality - Country	Coordinates	Years sampled	Breeding season months	Breeding events, individuals (males, females)
P/U-B	Snowy plover (*Charadrius nivosus*)	Ceuta - Mexico	23° 54′ N, 106° 57′ W	2008-2012	April-June	348, 181 (98, 83)
P/U-B	Kentish Plover (*Charadrius alexandrinus*)	Tuzla - Turkey	36° 43′N, 35° 03′E	1998-2000, 2004	April-June	260, 205 (97, 108)
P/U-B	Kittlitz's Plover (*Charadrius pecuarius*)	Andavadoaka - Madagascar	-22° 05'S, 43° 15'E	2012-2015	March-June	113, 94 (52, 42)
M/B	Kentish plover (*Charadrius alexandrinus*)	Maio - Cape Verde	15° 0.9′ N, 23° 12′ W	2009-2015	September-November	467, 233 (113,120)
M/B	Madagascan plover (*Charadrius thoracicus*)	Andavadoaka - Madagascar	-22° 05' S, 43° 15' E	2013-2015	March-June	38, 33 (18, 15)
M/B	White-fronted plover (*Charadrius marginatus*)	Andavadoaka-Madagascar	-22° 05' S, 43° 15' E	2013-2015	March-June	96, 74 (33, 41)

To assess how arrival dates and breeding schedule length differ between males and females among populations we constructed separate LMMs for each population. These mixed models allowed us to add the individual's identity as a random factor to control for repeated appearances of individuals throughout the years of study. For the Madagascan plover (Table 1) there were only 4 individuals out of 33 which had repeated breeding events so in this population a linear model was carried out instead using only the first breeding event of each individual. For each model we included sex as main term and total nests, relative body size, and minimum age as covariates. Two-way interactions of interest were sex * minimum age and sex * relative body size. The year of study was omitted from both analyses described above since this variable used three to seven degrees of freedom and there was no indication that there would be differences among years.

From saturated LMMs including all explanatory variables and two-way interactions of interest mentioned above, nonsignificant interactions and terms were sequentially backwards eliminated starting with least significant terms until minimal adequate models were reached (Crawley 2003). We report the significance of likelihood ratio tests (or Chi-squared tests for change in deviance in LM of Madagascan plover) comparing nested models with and without each variable and the β ± s.e. for all variables retained in the final models (we report back-transformed β ± s.e. for square root transformations). Goodness of fit of all mixed models was assessed using residuals versus fitted values plots and QQ plots of the residuals, boxplots of residuals by group and scatter plots of the estimated random effects to assess the within-group and random effects assumptions (Pinheiro and Bates 2004). All analysis were carried out in R (version 3.2.5) using the 'nlme' packages (R Core Team 2016).

Results

Arrival date

A marginally significant interaction between breeding system and sex was found in the multipopulation analysis suggesting that males and females may arrive at different times depending on the breeding system of the population (Table 2). Overall, males arrived 0.04 standard deviations (s.d.) earlier than females (from 0.5 to1.0 days difference depending on population, see s.d. for each population in Table 2) when controlling for individual's size (tarsus length) and the number of nests it produced (Table 2, Figure 1a).

Table 2. Arrival date and breeding schedule of male and female plovers in relation to breeding system, sex, body size and age in six plover populations using ID nested in population as a random factor in LMM. β ± s.e. are shown for variables and interactions kept in final models, L. Ratio and P values are shown for all variables and correspond to likelihood ratio tests from model simplification.

Variables	df	Arrival date		Breeding schedule	
		β ± s.e.	L.Ratio (P)	β ± s.e.	L.Ratio (P)
Intercept		2.41 ± 0.02		-0.46 ± 0.08	
Sex (males)	1	-0.04 ± 0.01	6.41 (0.01)	0.02 ± 0.08	8.22 (<0.01)
Sex x minimum age	1		0.05 (0.81)		0.17 (0.68)
Breeding system (polygamous)	1		0.18 (0.67)	-0.08 ± 0.08	0.10 (0.75)
Breeding system x sex	1		3.27 (0.07)	0.25 ± 0.11	4.08 (0.03)
Relative tarsus length	1	0.02 ± 0.00	5.10 (0.02)		3.37 (0.07)
Mínimum age	1		0.09 (0.76)		2.96 (0.08)
Total nests[ŧ]	1	-0.11 ± 0.01	66.89 (<0.001)	0.57 ± 0.05	103.71 (<0.001)

[ŧ]Total nests each individual had each breeding season.

Table 3. Mean ± s.d. of arrival date and breeding schedule for males and females of six plover populations. Sample sizes for each population in Table 1. Arrival dates are shown in Julian date (number of days since the 1st of January), breeding schedule is given in days.

Population	Arrival date [Julian date]		Breeding schedule [absolute number of days]	
	Males	Females	Males	Females
Snowy	106.11 ± 25.53	109.26 ± 25.45	74.94 ± 27.12	66.01 ± 28.52
Kentish - Tuzla	113.49 ± 15.54	116.89 ± 17.18	48.54 ± 19.98	44.37 ± 19.84
Kittlitz's	66.02 ± 18.26	71.44 ± 16.75	47.73 ± 15.99	47.04 ± 18.46
Kentish - Maio	257.01 ± 14.48	258.15 ± 15.47	59.57 ± 20.20	58.51 ± 20.32
Madagascan	67.00 ± 20.01	68.41 ± 11.37	61.00 ± 29.67	54.35 ± 15.19
White-fronted	71.22 ± 18.00	68.08 ± 13.95	55.50 ± 22.68	57.78 ± 22.30

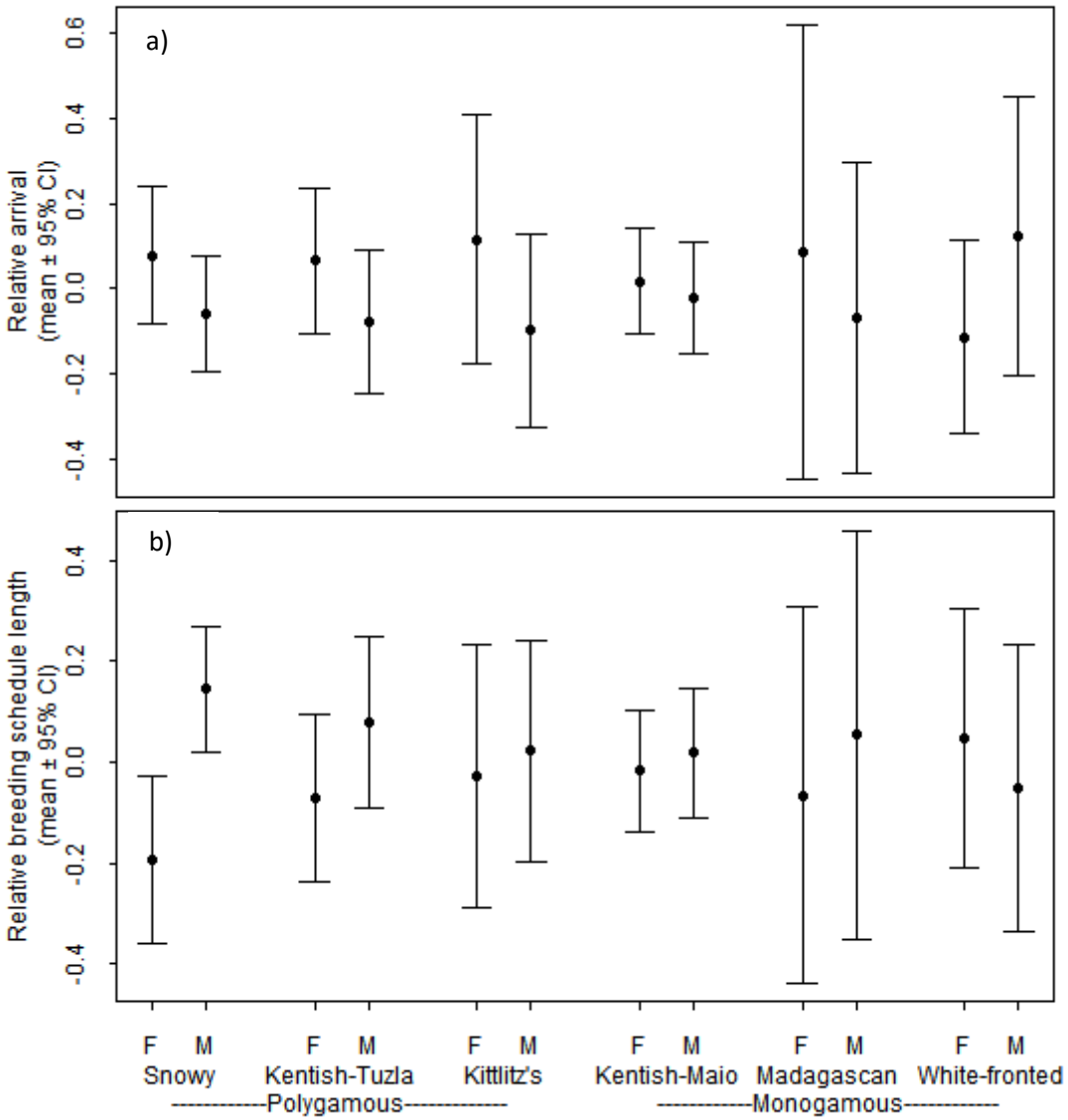

Figure 1. a) Arrival date ± 95% CI and b) breeding schedule ± 95% CI for females and males of six plover populations

Single-population models indicated that the arrival dates of males were not significantly different from those of females except in the snowy. Males from the snowy plover population arrived significantly earlier than females to the breeding grounds (Table S1, Table 3 and Figure 1a) after controlling for the number of nests each individual produced.

Body size was significantly correlated with arrival dates in Kentish plovers in Maio, and in white-fronted plovers: large individuals arrived later than smaller individuals in both sexes (Table S1). In the Madagascan plover, older females tended to arrive later than younger ones, but in males this pattern was the opposite (Table S1).

Breeding schedule

A significant interaction between breeding systems and sex was found in the multipopulation analysis (Table 3), revealing that males had longer breeding schedules than females in polygamous populations, whereas in monogamous populations there was no sex-difference in breeding schedule (Table 3, Figure 1b) when controlling for the number of nests individuals produced.

Single-population modelling indicated that the breeding schedules of males and females were not different in any of the populations, even after controlling for the number of nests each individual produced, with the exception of the snowy plover (Table S1, Table 2). Within the snowy plover population, males stayed significantly longer on the breeding grounds than females (Table S1, Table 2 and Figure 1b).

In the Kentish plover of Maio, larger individuals had shorter breeding schedules regardless of their sex and the number of nests each individual produced (Table S1). In all of the plover populations and species studied with the exception of the Madagascan plover, individuals who produced more nests had longer breeding schedules (Table S1).

Discussion

Our two main results were consistent with our predictions. Firstly, males tended to arrive earlier than females in polygamous populations but not in monogamous ones ($P = 0.07$). Secondly, males had significantly longer breeding schedules than females but only in polygamous populations. Curiously, differences between monogamous and polygamous populations seemed to be driven by a single population, the snowy plover.

Arrival date

In line with our predictions, males arrived earlier than females only in polygamous populations. Our multipopulation model showed a marginally significant interaction between breeding system and sex, but the population models showed that the snowy plover was the only polygamous population exhibiting a significant difference in arrival date between males and females.

In birds, protandry has been studied mostly in sexually dimorphic species that exhibit normal sex-roles and also in socially monogamous species with biparental care (reviewed in: Morbey et al. 2012). However, important differences between the above species and the monogamous species that we have studied can be found in the amount of sexual dimorphism and the incidence of extra-pair paternity. Male and female plovers from

monogamous populations are similar in size (males are slightly larger than females), weight, and ornamentation (Argüelles-Ticó et al. 2016). While 90% of bird species are monogamous, extra-pair paternity is prevalent in a large proportion of monogamous avian species (affecting on average over 11% of offspring, Griffith et al. 2002), extra-pair paternity is rather uncommon in plovers (Küpper et al. 2004, Maher et al., submitted). Sexual dimorphism and high incidence of extra-pair paternity are the typical indicators of strong sexual selection and mate competition (Andersson 1994; Dunn et al. 2001). In line with this, protandry tends to be more common in species with high levels of extra-pair paternity (Rubolini et al. 2004; Coppack et al. 2006). Sexual selection and mate competition may not be very strong in most of the monogamous plover species studied, so it is reasonable that we found no evidence of protandry since males do not need to arrive earlier to outcompete other males or attain any other of the potential benefits hypothesized (Morbey et al. 2012).

Explaining the lack of a statistically significant protandry is more complex for the three polygamous populations in our study (snowy, Kentish-Tuzla, Kittlitz's), as only one of these populations showed protandry. We suggest two explanations for this finding. Firstly, snowy plovers have a highly male-biased ASR (Carmona-Isunza et al. in review, Eberhart-Phillips et al. in prep); and from preliminary comparisons of ASRs from these six populations it seems that the snowy plover has the most extreme male-biased ASR (Eberhart-Phillips et al. in prep). If the adult sex ratio is extremely male-biased, males that arrive late will have extreme difficulty finding an unmated female. However if the ASR is not extremely male-biased as in the Kentish plover in Tuzla or in the Kittlitz's plover, males might not benefit as much from arriving early since females re-mate throughout the breeding season and thus offer these males the opportunity for later matings.

Secondly, even if these three populations are considered polygamous there are slight differences among them that may be important for protandry. For example, the breeding system in the Kittlitz's plover is somewhat complex. Preliminary analysis revealed high mate fidelity (Harding et al. in prep), however, the mating opportunities appear to differ for male and female Kittlitz's plovers since males find mates faster than females and spend less time courting (Parra et al. 2014). Curiously, Kittlitz's plover broods are tended uniparentally by males (Eberhart-Phillips, unpublished data), leaving females able to re-mate. This portrays a scenario in which females could potentially experience higher mating competition than males and would have to arrive early to find a mate, however as males have limited time to find a mate since they will spend a significant larger amount of time caring for a brood than females they would as well benefit from arriving early to breed. Therefore in the Kittlitz's plover it may be beneficial to arrive early to the breeding site regardless of the sex.

In sex-role reversed species, females usually arrive before males (protogyny, Oring and Lank 1982; Reynolds et al. 1986). Although snowy plovers are considered to exhibit sex-role reversal we did not predict protogyny because of the highly male-biased ASR present in the snowy plover (Carmona-Isunza et al. in review, Eberhart-Phillips in prep) which translates into a higher mate competition among males than among females as male snowy plovers spend a higher proportion of time courting than females (Carmona-Isunza et al. 2015).

The populations we studied also exhibit variable migratory patterns. While two of the localities are islands (i.e. Maio and Andavadoaka) and the plover populations that breed there are considered residents, some individuals do not stay on the breeding grounds throughout the whole year (around 43%, 32% and 21% of colour ringed individuals that bred each year were not seen during non-breeding season surveys of that year in Maio respectively throughout 2013, 2014 and 2015, unpublished data). Snowy plovers are considered to be partial residents as some individuals do stay close to the breeding grounds the whole year (Colwell et al. 2007; Carmona-Isunza et al. 2015) while Tuzla's Kentish plover population is considered migrant (Sandercock et al. 2005). Thus, it is possible that the contrasting migratory patterns between the populations studied may have influenced our results. Consequently, further multipopulation studies using additional populations with variable migratory patterns are needed to understand how these could potentially influence protandry.

Although we used data from opportunistic re-sightings (all populations) and breeding season surveys (in the the snowy and Kentish from Maio), nesting data was also very important to know the arrival dates of individuals. A potential limitation of relying in nesting data is that given that males and females start nesting at the same time their arrival times would be the same. Moreover, re-sighting effort between populations may have been different given that in the snowy plover population and in the Kentish from Maio besides re-sightings, surveys were carried out throughout the breeding season every two weeks or every week. Although it is possible that the difference in re-sighting effort may have led us to find small differences between males and females in populations where no survey effort was done (Kentish-Tuzla, Kittlitz's, Madagascan and white-fronted), the proportion of arrival dates that came from re-sightings or surveys were similar throughout all populations and sometimes higher in populations were no surveys were done (snowy: 33% of 348 observations, Kentish-Tuzla: 41% of 260, Kittlitz's: 25% of 113 , Kentish-Maio: 31% of 467, Madagascan: 63% of 38, white-fronted: 50% of 96). Therefore we do not think it was possible that differences in re-sighting effort could have biased our results.

Breeding schedule

In line with our predictions, males had longer breeding schedules than females in the polygamous populations only. Our multipopulation model revealed a significant interaction between breeding system and sex. However, the single-population models showed that the snowy plover was the only polygamous population showing a difference in breeding schedules between males and females.

A breeding schedule is delimited by an individual's arrival and departure date. Therefore, our discussions about variation in arrival date are closely tied to the patterns we observed in breeding schedules. Departure date depends principally on nesting success, chick survival, and the sex which provides brood care and re-mating opportunities. In monogamous populations with biparental care, breeding pairs may remain together for several nesting events in one season if a nest fails and will care for a brood together until fledging. In contrast, breeding pairs in polygamous populations remain together during incubation (around 20 days), after which point females (snowy, Kentish – Tuzla, Kittlitz's) may abandon the brood. In the snowy plover it is primarily the males who take care of broods and brood care takes between 20-25 days until fledging occurs. Even if females mate twice, two periods of incubation may be shorter than one successful breeding event of an abandoned male.

The difference we found between the snowy plover (males had longer breeding schedules than females) and the Kentish plovers from Tuzla (male and female breeding schedules did not differ) may depend partly on interspecific variation in chick survival. Chick survival in Tuzla was demonstrated to be comparably better if both parents care for chicks rather than one (Székely and Cuthill 1999), whereas in the snowy this difference is likely to be negligible, although studies would be needed to confirm this. Therefore, females in Tuzla may be more likely to stay with their chicks in their second breeding attempt to increase chick survival and have a comparable breeding schedule to that of males, while in the snowy plover females may desert broods for all breeding attempts. A fruitful avenue for future study may be to evaluate sex-specific fitness of shorter versus longer breeding schedules in the context of chick survival in populations exhibiting biparental care and uniparental care. Kittlitz's plover has been proposed to exhibit a flexible breeding system since males care for broods (Eberhart-Phillips, unpublished data) and females confront high mating competition (Parra et al. 2014), it is possible that mean breeding schedules are comparable between sexes as males are caring for broods while females are trying to re-mate, spending a similar amount of time in the breeding grounds.

Implications of breeding schedules for sex ratios

Sex-differences in breeding schedule may have important demographic implications. In particular, ASR is a key demographic property of a population and recent studies suggest that ASRs can be highly skewed in wild populations (Donald 2007; Székely et al. 2014). Notably, these skews have important implications for both breeding system evolution and biodiversity conservation (Donald 2007; Liker et al. 2014). Biased ASRs may arise from a biased sex ratio at birth, sex-differential mortalities of young and adults, sex-differential maturation times, and sex-differential dispersal and migration patterns (detailed in Székely et al. 2014). Sex-specific differences in breeding schedule duration provide a further potential source for variation in ASRs that remains virtually unexplored so far (but see Payne et al. 2011). For example, male-biased ASR estimations could arise in surveys if males remain longer in the breeding grounds than females, even if the population ASR is not biased.

Understanding breeding schedules is also important in the context of operational sex ratio (OSR, proportion of sexually active males in the adult population), especially for species that have multiple breeding attempts in a single season. The duration of time-in and time-out periods (sexually active and inactive periods respectively; Clutton-Brock and Parker 1992; Székely et al. 2000; Alonzo 2010) will depend on the total time an individual spends on the breeding grounds and this may directly impact OSR. A good understanding of breeding schedules is therefore important for studies evaluating both ASR and OSR.

In conclusion, our study reveals that protandry and sex differences in breeding schedules may depend on the breeding system of a population. We have highlighted the demographic implications of these findings and and their potential impacts on ASR and/or OSR. Further multipopulation studies with other, more diverse taxa with varying breeding systems (e.g. frogs, fish, and other avian species) will help to understand better the role of breeding schedules and protandry in breeding systems evolution.

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Table S1. Model simplification results from each population's LMMs for i) arrival date and ii) breeding schedule length using individuals id as a random factor. β ± s.e. are shown for variables and interactions kept in final models, L. Ratio and P values are shown for all variables and correspond to likelihood ratio tests from model simplification.

Variables	df	Snowy		Kentish-Tuzla		Kittlitz's		Kentish-Maio		Madagascan*		White-fronted	
		β ± s.e.	L.Ratio (P)	β ± s.e.	L.Ratio (P)	β ± s.e.	L.Ratio (P)	β ± s.e.	L.Ratio (P)	β ± s.e.	Dev. (P)	β ± s.e.	L.Ratio (P)
i) Arrival date													
Intercept		1.04 ± 0.18		0.46 ± 0.17				0.50 ± 0.13		-0.85 ± 0.40		0.44 ± 0.25	
Sex (males)	1	-3.99 ± 0.00	5.90 (0.01)		1.79 (0.18)		1.85 (0.17)		1.21 (0.28)	-3.92 ± 0.08	0.00 (0.99)		0.00 (0.98)
Sex x minimum age	1		0.00 (0.96)		0.00 (0.99)		0.00 (0.98)		0.21 (0.64)	-3.98 ± 0.05	0.25 (0.03)		0.05 (0.82)
Sex x relative tarsus length	1		1.78 (0.18)		0.19 (0.66)		2.06 (0.15)		0.00 (0.94)		0.00 (0.79)		0.02 (0.88)
Relative tarsus length	1		0.46 (0.50)		2.44 (0.12)		0.23 (0.63)	-4.00 ± 0.00	4.27 (0.04)		0.07 (0.28)	-3.99 ± 0.00	7.40 (<0.01)
Minimum age	1		1.10 (0.29)		0.41 (0.52)		0.00 (0.92)		0.82 (0.36)	-3.99 ± 0.00	0.06 (0.32)		0.03 (0.86)
Total nests**	1	-3.98 ± 0.01	34.75 (<0.001)	-3.99 ± 0.00	11.53 (<0.001)		2.24 (0.13)	-3.99 ± 0.00	22.39 (<0.001)		0.00 (0.74)	-3.99 ± 0.00	4.210.04)
ii) Breeding schedule length													
Intercept		-1.60 ± 0.21		-0.58 ± 0.16		-0.85 ± 0.41		-0.16 ± 0.19				-0.49 ± 0.26	
Sex (males)	1	1.13 ± 0.28	20.85 (<0.001)		1.97 (0.16)		0.29 (0.59)		1.33 (0.25)		0.06 (0.75)		0.02 (0.89)
Sex x minimum age	1		0.06 (0.80)		1.44 (0.23)		0.44 (0.50)		0.03 (0.86)		2.03 (0.06)		0.28 (0.87)
Sex x relative tarsus length	1		1.59 (0.20)		2.38 (0.12)		0.92 (0.33)		1.30 (0.25)		0.12 (0.66)		0.52 (0.47)
Relative tarsus length	1		0.11 (0.74)		0.03 (0.87)		0.02 (0.88)	-0.11 ± 0.05	6.01 (0.01)		0.00 (0.93)		3.52 (0.06)
Minimum age	1		6.57 (0.01)		0.72 (0.40)		0.04 (0.85)				0.46 (0.38)		0.60 (0.44)
Total nests**	1	1.09 ± 0.13	78.24 (<0.001)	0.48 ± 0.12	13.72 (<0.001)	0.79 ± 0.38	3.76 (0.05)	0.58 ± 0.09	36.65 (<0.001)		1.45 (0.12)	0.42 ± 0.21	3.81 (0.05)

*Madagascan plover models shown are LMs using the first observation of each individual, as not enough repetitions were available for mixed modesl (n = 33 individuals).**Total nests each individual had each breeding season

6 Discussion

Understanding breeding system evolution has been a central topic in evolutionary biology and behavioural ecology (Emlen and Oring 1977; Reynolds 1996; Ligon 1999; Royle et al. 2012; Arnqvist and Rowe 2013). Empirical studies that compare ecological, demographical and behavioural aspects between populations with diverse breeding systems are essential to understand how and why different breeding systems arise. The main objective of my dissertation is to understand how ASR, a key component of the social environment, is related to breeding systems. In order to address this question, I use plovers *Charadrius* spp – a group of species that exhibit diverse breeding systems – in a multi-population approach. My dissertation goes beyond previous studies of breeding systems of plovers in two respects:

(i) Unlike previous studies that have focused on abiotic environments and life-history traits such as environmental harshness and brood size (AlRashidi et al. 2010; AlRashidi et al. 2011; Vincze et al. 2013; reviewed in Székely 2014), my work is focused on the social environment using ASR as a proxy variable;

(ii) The results are built upon decades of data collection in six plover populations (Székely and Cuthill 1999; Székely et al. 1999; Kosztolányi et al. 2006; Parra et al. 2014; Carmona-Isunza et al. 2015b), and I use the natural variation in mating and parenting behaviour and number of males and females to explore relationships between breeding systems and ASR.

Although previous studies have characterised both the mating system and parental care of these populations (Argüelles-Ticó 2011), comprehensive quantitative reviews of both mating and parenting are underway (Harding et al. in prep, Seymour et al. in prep). In addition, a demography-based ASR estimation of six plover populations is underway (Eberhardt-Phillips et al. in prep).

My dissertation provides four main results: i) ASR is unrelated to OSR in a polygamous plover population (Chapter 2); ii) courtship rate is higher in a polygamous plover population compared to a monogamous one (Chapter 3); iii) ASR is a better predictor of female care than OSR in a population where females have variable duration of care (Chapter 4); iv)

males arrived earlier to the breeding grounds and stayed longer than females in the polygamous population with an extremely male-biased ASR, whereas in polygamous populations with a more moderate bias in ASR and in monogamous populations the arrival times of males and females did not differ (Chapter 5).

ASR and OSR refer to different subsets of animals (the adult population altogether or the adult population that is reproductively active respectively) but they have often been confused and used interchangeably (Kokko and Jennions 2008; Székely et al. 2014). Even if ASR and OSR are closely related, as one is the precursor of the other, it is important to understand under which conditions they can be identical or different. I found that OSR was highly variable over the breeding season while ASR was not, this is a fundamental aspect of sex ratios that needs to be considered. Although ASR may also vary over time as it depends on sex-specific mortalities, maturation or migration-emigration (Székely et al. 2014), given the origin of ASR this variation is in a different time scale than OSR's variation, i.e. over a period of time longer than days or weeks. The difference in time variability may have been the cause for the lack of correlation between ASR and OSR. Recent empirical and theoretical studies suggest that ASR may be an important driver for breeding system evolution (Kokko and Jennions 2008; Jennions and Kokko 2010; Liker et al. 2013; Liker et al. 2014a). However, the intricate and unexplored feedbacks between ASR and breeding systems may imply that breeding system is also important in sculpting the relationships observed between ASR and OSR, as the relationship between ASR and OSR may differ in populations with different breeding systems. I examined the association between ASR and OSR in a single polygamous population with a highly male-biased ASR, but it may be relevant to carry out similar studies evaluating the correlation of fine scale temporal estimates of ASR and OSR on different populations with varying breeding systems. For example, in a monogamous population with biparental care ASR and OSR are expected to be correlated: in principle ASR would be unbiased, pairs would stay together throughout the breeding season so one would expect that OSR would also be unbiased as few individuals remain unmated. Future studies of both ASR and OSR using populations with variable breeding systems will be valuable since they will help to disentangle the feedbacks between ASR, OSR and breeding systems.

The intensity of sexual selection and the extent of ornamentation are often assumed to be related to the degree of polygamy (Møller and Pomiankowski 1993; Lindenfors and Tullberg 1998; Baker and Wilkinson 2001; Dunn et al. 2001; Price and Whalen 2009). However, studies evaluating the relationship between the degree of polygamy and courtship behaviour were lacking. I found that a polygamous population had higher rates of courtship than a

monogamous population (Chapter 3). Although this was based on a comparison between two closely related plover species, it provided empirical evidence to the assumed but untested idea that courtship behaviour would be higher in polygamous than monogamous populations. A likely explanation to this result is the difference between ASR that exists in both populations. ASR can be related to the degree of polygamy or monogamy observed in a population, as a comparative study using shorebirds suggests that male-biased ASRs predict polygamy whereas unbiased ASRs predict monogamy (Liker et al. 2013). Consistently with this logic, the polygamous population in our study had a male-biased ASR (Chapter 2) and the monogamous population had an unbiased ASR as confirmed by a provisional analysis of surveys (see details in discussion of Chapter 4). The social environment was one of the most conspicuous difference between the populations compared in that study, and given that the breeding system is not species-specific (e.g. Kentish plover in Maio, Cape Verde is monogamous, Kentish plover in Tuzla, Turkey is polygamous), the difference in the social environment (ASR) was considered the most likely explanation for the differences in courtship behaviour. It will be interesting to evaluate populations with different biases in ASR and see whether courtship rates will respond to increasingly male-biased ASRs.

Biased OSR's have been thought of as the unequivocal precursors for high mate competition and sexual selection intensity that may create the environment for polygamy (Emlen and Oring 1977; Shuster 2016). Emlen and Oring (1977) in their seminal paper even state "*To understand the intensity of sexual selection it is not the overall population ratio of males to females that is of importance but rather what we term the operational sex ratio (OSR)…*". However, empirical studies testing the association between the intensity of sexual selection and OSR have found contrasting results. For example, biased OSRs may intensify sexual selection in arthropods (Jann et al. 2000; Foellmer and Fairbairn 2005), fish (Balshine-Earn 1996; Jones et al. 2001) and frogs (Emlen 1976), but decrease intensity of sexual selection in crustaceans (Shuster et al., 2001 as cited in Shuster 2016), and did not affect sexual selection in whales (Cerchio et al. 2005). New theory and comparative studies highlight that it is ASR which may be more important for breeding systems than OSR (Kokko and Jennions 2008; Jennions and Kokko 2010; Liker et al. 2013; Liker et al. 2014a). ASR may be a better predictor for mate competition than OSR since (i) OSR is more variable and less predictable than ASR, given that OSR estimations depend on the number of males and females that enter and exit the breeding pool, and (ii) OSR can be viewed as a reflection of a breeding system rather than the driver of it. ASR is expected to be more constant than OSR because in a breeding season the only factors that may impact ASR are migration, emigration and mortality, which are expected to be less variable throughout a breeding

season than number of males and females entering and exiting the breeding pool. Therefore, ASR may be more "visible" to individuals in wild populations and more reliable as a cue to assess mate competition. Following this logic I proposed (Chapter 4) that ASR should be better than OSR at predicting female's duration of care in a plover species where females may desert their brood to mate with other males, and the data supported my prediction. I argued that ASR predicts better female parental behaviour than OSR because relative stability of ASR may provide more reliable information about costs (i.e., less breeding events, lower chick survival) and benefits (i.e., higher chick survival, increased breeding events) of staying with the current brood versus abandoning it and mating with additional partners. This provides, to my knowledge, the first empirical evidence that ASR predicts parental care better than OSR. More empirical studies in different taxa considering the dynamic nature of ASR and OSR are warranted to further confirm the generality of these findings. It will be interesting to test this prediction with a multi-population approach evaluating other aspects of breeding and testing if ASR or OSR are better cues to signal mate competition or other costs and benefits of mating decisions.

In Chapter five, I show that males arrive earlier than females to the breeding grounds and had longer breeding schedules in a polygamous population with extreme ASR but not in polygamous populations with moderately biased ASRs or in monogamous populations. Breeding schedules are tightly linked to the type of breeding system seen in a population because males and females could make a similar or different investment in mate competition, breeding behaviours and parental care depending on the breeding system. For example, in monogamous populations with biparental care males and females are expected to have more similar breeding schedules as both sexes spend similar times looking for mates, courting, breeding and caring for their offspring compared to a polygamous population with uniparental care. The relevant aspect of sex-specific duration of breeding schedules is that they can have demographic implications over the ASR in a population. Although biased ASRs arise from a biased sex ratio at birth, sex-differential mortalities of young and adults, sex-differential maturation times, and sex-differential dispersal and migration patterns (detailed in Székely et al. 2014), a potential source for variation in ASRs, unconsidered so far, are the sex-specific different durations of breeding schedules (but see Payne et al. 2011). For example, even if the population ASR is not biased, male-biased ASR estimations could arise from surveys if males remain longer in the breeding grounds than females, or vice versa. It will be interesting to compare survey-based estimates of ASR with demographic model estimates (e.g., Veran and Beissinger 2009; Kosztolányi et al. 2011) to see whether a more extreme bias is seen in survey-based estimates of populations with sex-specific breeding schedules. Sex differences in breeding schedules may also affect OSR

estimates. The total time an individual spends in the breeding grounds will influence the duration of time-in and time-out periods (sexually active and inactive periods respectively; Clutton-Brock and Parker 1992b; Székely et al. 2000; Alonzo 2010) and this may directly impact OSR. A good understanding of breeding schedules is therefore important for both ASR and OSR.

This dissertation emphasised the roles of social environment in driving breeding systems by addressing several aspects of breeding systems: the social environment (Chapter 2), courtship (Chapter 3), parental care (Chapter 4) and timing of breeding (Chapter 5) in multiple populations with varying breeding systems. The results presented in this dissertation help to clarify aspects of breeding systems that had been neglected or assumed, such as differences in courtship behaviour and in the time spent breeding between monogamous and polygamous species. Moreover, this dissertation provided empirical evidence of the importance of ASR over OSR in one aspect of breeding systems: parental care. Also, I presented the first formal empirical evidence that ASR and OSR are not correlated and behave differently throughout the breeding season. In conjunction these results tell us that the relation between the social environment and breeding systems is intrinsic. A breeding system describes the mating strategies: form of courtship and competition, number of mates, duration of pair bonds and the post-mating behaviours like the form and duration of parental care (Reynolds 1996), and each of these may be influenced by the social environment present in the population (see figure 1 in Chapter 1). Moreover, the social environment varies through time and should not be considered fixed.

An important limitation of this study and any study evaluating ASR and OSR is the way in which sex ratios are estimated. Several methods for estimating ASR and OSR exist (e.g. survey based, via demographic models, genetic analysis of carcasses or faecal droppings), each of these has pros and cons which need to be considered based on the objectives of the study. Importantly, the individual-based method we implemented in our study to estimate ASR and OSR allowed us to observe daily variations, which no other method allows. Moreover, average ASR estimations from our study in the snowy plover in Ceuta seem to be consistent with preliminary results from a demography-based ASR estimation project that used snowy plovers in Ceuta plus five more plover populations (Eberhardt-Phillips et al. in prep). Another important limitation of the studies presented here is that these are correlational, since ASR and breeding systems are intrinsically related it is not possible to understand their effects separately as it would with experimental studies.

Future directions

Although the study of breeding systems and its evolution has been fruitful, our understanding is still limited. Moreover, the role of the social environment in breeding system evolution seems to be still fixed on OSR. It is striking that even with the plethora of recent studies stressing ASR's importance (Donald 2007; Kokko and Jennions 2008; Jennions and Kokko 2010; Székely et al. 2014) a recent review about the importance of OSR failed to acknowledge ASR's importance or even mention it (e.g. Shuster 2016). In order to make further progress in this field I suggest six avenues:

1. In future studies of sexual selection, mating competition and breeding system, considering both ASR and OSR will need to be a fundamental part. Moreover, as recent models suggest, it may be relevant to acknowledge the source of variation of ASR and the sex ratio at maturity given that they can also account for variation in parental care (Fromhage and Jennions 2016).
2. More holistic studies that compare mating behaviour, parental care, reproductive success, survival and different measures of the intensity of sexual selection (e.g. Bateman gradient, opportunity for selection, standardized mating differential, Jones 2009) among populations with varying breeding system will prove necessary to understand variation in ASR, OSR, sexual selection and their link to breeding systems. These studies will become more likely as data from long-term monitored populations keeps accumulating.
3. One fruitful avenue for future research would be to compare how sex-specific mortalities, sex-difference in migration patterns and sex-specific maturation times contribute to variation in ASR biases across populations with different breeding systems. Under different breeding systems the costs from maturing early, mating or caring may differ and contribute in different ways to variation in ASR. For example under polygamy the sex experiencing higher mating competition may suffer higher mortality biasing the ASR towards the sex with lower mating competition. On the other hand under monogamy the sexes are expected to experience similar levels of mating competition, and therefore biases in ASR will be due to other sex-specific differences in maturation times, migration patterns or mortalities not necessarily related to mating competition. Understanding the source of ASR biases under different breeding systems will help disentangle part of the feedbacks between the social environment and breeding system evolution.
4. Addressing the feedbacks between breeding systems, ASR and OSR will prove a fruitful avenue in the near future. Although difficult to implement, experimental studies

attempting to manipulate the ASR in a monogamous and a polygamous wild population and then evaluate responses in terms of mating competition, courtship behaviour and parental care would be the best way to understand the causal relation between ASR and breeding systems and their possible feedbacks. Aside from obtaining field permits, one of the difficulties in implementing ASR manipulations is that one cannot subtract/add adult males or females in a population without manipulating OSR at the same time (as one would subtract/add adult males or females which are potentially breeders). It could be possible to manipulate ASR independently from OSR if some breeders are experimentally constrained from breeding (e.g. using hormones or some kind of chastity belts), however it is unknown if their behaviour would be modified or if these individuals would be considered available or non-available by other individuals. Another possibility is to manipulate OSR prolonging or shortening time-out periods, for example in birds, replacing offspring constantly in some pairs so that parents will provide care throughout a longer period and/or kidnapping offspring from some pairs to make them available to breed again. By manipulating time-out periods it would be possible to modify ASR independently from OSR, for example by kidnapping caring males but leaving their paired females to provide parental care for longer (by replacing offspring), however this would only work in a population where both parents provide care and it is possible for a single parent to raise a brood successfully.

5. Understanding the mechanisms through which the social environment influences individual's behaviour may prove interesting. Using new technics in genomics and neuroscience will offer important insights into the way the social environment influences parental behaviour and mating behaviour (O'Connell and Hofmann 2012).
6. Shorebirds, the avian group in which plovers belong have proved to be important to study breeding system evolution (Szekely and Reynolds 1995; Thomas et al. 2007), but fish, frogs and other bird groups that show substantial variation in breeding systems have also provided insightful results. It will be an important avenue in future research to include these taxa in experimental and phylogenetic comparative studies

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In conclusion, the social environment is central to understanding breeding systems and breeding system evolution. As theoretical models keep improving and adapting new insights empirical studies will have to follow so that our understanding of this exciting field progresses. This field will benefit much from experimental studies, long-term studies of wild populations and phylogenetic comparative analysis.

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Appendix I

Morphological and genetic differentiation among Kentish Plover Charadrius alexandrinus populations in Macaronesia

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This paper reports on original research I helped to conduct during the period of my Higher Degree by Research candidature.

Author contributions

MA: molecular analysis, statistical analysis, manuscript preparation
KK: statistical analysis, manuscript preparation
MCC-I: data collection in the field, manuscript preparation and improvement
PL: data collection in the field
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AK: data collection in the field, manuscript improvement
TS: study conception and design, manuscript preparation
CK: study conception and design, manuscript preparation

Abstract

Macaronesia, a group of archipelagos (Azores, Madeira, Canary Islands and Cape Verde) in the Atlantic Ocean, is one of the global biodiversity hot-spots. However, for species that occur throughout these archipelagos the extent of genetic and morphological differentiation between the archipelagos and mainland Iberia and Africa is often unknown. Here we investigate phenotypic and genetic differentiation of Kentish Plovers Charadrius alexandrinus, the most common breeding shorebird of Macaronesia. We show strong genetic and moderate morphological differences among breeding populations. Our results suggest that although these populations are far from being a species-level endemism, they deserve conservation attention given their uniqueness in terms of genetic variation. Recent loss in suitable breeding sites in Canary Islands and Azores put substantial pressure on the extant plover populations. Further studies of the conservation status and threat to these populations are needed along with a comprehensive conservation action plan to halt population decline and facilitate recovery.

Key words: Kentish Plover, phenotypic divergence, genetic structure, Macaronesia.

Introduction

Divergence of populations may arise by limited dispersal and gradual genetic drift across populations (isolation by distance), adaptation to local environments preventing gene flow between populations (isolation by adaptation), or colonization history and founder effects (isolation by colonization, reviewed by Orsini *et al.,* 2013). Given the geographic isolation that may reduce exchange of migrants, island populations are more prone to speciation than mainland populations (Adler, 1992; Emerson, 2002), and have therefore been important study systems in which to investigate fundamental ecological and evolutionary processes such as population divergence, adaptive radiation and speciation (Warren *et al.,* 2015, Illera *et al.,* 2016).

Macaronesia is a collection of five archipelagos located in the North Atlantic Ocean off the coast of Europe and Africa. It includes Azores, Madeira, Selvagens, Canary Islands and Cape Verde. The Macaronesian Islands are an excellent study system to investigate evolution and radiation of various taxa including plants, reptiles and birds given their substantial variation in distances from mainland and between members of the various archipelagos, and the variations in geological age of different islands (Juan *et al.* 2000; Whittaker and Fernandez-Palacios, 2007; Illera *et al,.* 2007; Illera *et al*., 2012). The islands of Macaronesia have a unique biogeography and given their high habitat diversity they are home to a rich endemic biota (Izquierdo *et al.,* 2004; Arechavaleta *et al.,* 2005; Borges *et al.,* 2008, 2010), and are considered one of the global Endemic Bird Areas (Stattersfield *et al.,* 1998). Much of the avifauna of the Macaronesian Islands exhibits genetic differentiation between islands or archipelagos, suggesting that there is limited gene flow between islands providing the opportunity for population differentiation and eventually new species to arise (Pestano *et al.,* 2000; Dietzen *et al.,* 2003; Kvist *et al.,* 2005; Päckert *et al*., 2006; Illera *et al.,* 2007). Various processes, however, threaten the unique fauna and flora of these islands. Poaching and illegal killing of marine turtles, bycatch of dolphins and sharks, logging of forests for timber and firewood, clearing vegetation for grazing and agriculture, and the introduced exotic plants and animals by humans threaten the endemic plants and animals (Garzón-Machado *et al.,* 2010; Martín *et al.,* 2010; Fontaine *et al.,* 2011; Vasconcelos *et al.,* 2013; Dutra and Koenen, 2014). As a result, many of the endemic species of the islands are seriously endangered or extinct (Hazevoet, 1995; Martín *et al.,* 2010). Several studies documented that throughout the last million years island faunas have been gradually destroyed through the process of human disturbance, biological invasions and habitat exploitation (Seoane *et al.,* 2011; Illera *et al.,* 2012).

The Kentish Plover *Charadrius alexandrinus* is a widespread Eurasian shorebird species (Amat, 2003) that inhabits North Africa, Europe and Central and Eastern Asia (del Hoyo *et al.,* 1996). A previous study of genetic differentiation between different Kentish Plover populations (Küpper *et al.,* 2012) showed genetic differentiation at relatively modest distances (e.g., 40 km) between mainland and island breeding sites. Here we extend this study by focusing on genetic and morphological differentiation between Macaronesian and continental Kentish Plover populations.

This study has two aims. The first is to test for morphological and genetic differentiation among Kentish Plover populations across Macaronesian region. The second is to investigate whether morphological and genetic differentiation correlates with each other as well as with geographic distance. Clarifying the morphological and genetic differentiation between these populations is warranted for two reasons. First, the breeding Kentish Plover populations in some of these archipelagos (e.g. Canary Islands) are rapidly declining (Lorenzo and Barone, 2007). Such a circumstance could prompt the extinction of differentiated populations before being considered their protection. Second, morphological and genetic differentiation between different archipelagos and mainland would imply that conservation and management of their breeding site and protection of the remaining habitats are important given the uniqueness of these populations.

Methods

Sample collection

Morphometric measurements were collected between 1994 and 2013 during the breeding season by a total of 16 observers. Birds were caught at different localities from islands at four archipelagos (Table 1): i) Cape Verde (Boa Vista and Maio), ii) Azores (Santa Maria), iii) Canary Islands (Fuerteventura), and iv) Madeira (Porto Santo). In addition, we sampled mainland populations in two continental areas in Europe (Portugal: Samouco Salt Pans, Fuseta Salt Pans), and Africa (Morocco: Oued Gharifa Salt Pans) (Table 1, Figure 1). In Fuseta only morphological data were collected. The samples were collected using consistent methodologies. Adult plovers were caught using mist-nets or funnel traps whilst they incubated the nest or attended the chicks (Székely *et al.,* 2008). All birds were ringed with uniquely numbered metal rings, and two morphological traits were measured for each adult: 1) right wing (to the nearest mm), flattened and straightened from the carpal joint to the tip of the longest primary feather; 2) right tarsus (to the nearest 0.1 mm), from the notch of the knee to the tarsus bone ends. Blood samples were collected by brachial venipuncture and stored in either Queen's Lysis Buffer (Seutin *et al.,* 1991) or pure ethanol and kept at 4°C

(Queen's Lysis Buffer samples) or room temperature (ethanol samples) until DNA extraction. Birds were released at their capture locations.

Morphological differences between populations

Wing length and tarsus length between sex and populations were analysed using two-way ANOVAs and Tukey HSD tests. The homogeneity of variance for the morphometric data for each sex was tested with the Bartlett's test (Snedecor and Cochran, 1989), and normality was assessed by a Shapiro-Wilk test (Shapiro and Wilk, 1965). Both tests suggested that the morphometric data had homogeneous variances and were not different from normal distribution (all $P > 0.05$). Because multiple observers measured the phenotypic traits, differences could perhaps be explained due to observer effects. To investigate this we tested for differences between archipelagos using data from two independent observers that had sampled at least two archipelagos. We found qualitatively similar differences between archipelagos (data not shown) as with using the full data set; hence we feel confident in the validity of our approach.

To assess morphological differentiation we calculated pairwise phenotypic distances (P_{ST}) between breeding locations for males and females separately. The P_{ST} index can be interpreted similarly to the commonly used F_{ST} index obtained from neutral genetic markers (Saint-Laurent *et al.,* 2003; Raeymaekers *et al.,* 2007). P_{ST} values close to zero indicate similarity between phenotypes whereas increasing positive values point towards high dissimilarity between phenotypes. P_{ST} values of each trait were calculated separately for males and females between all population pairs using statistics derived from one-way ANOVAs, and P_{ST} was assessed as $P_{ST} = \sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$ as in Sokal and Rohlf (1995), where σ^2_{GB} and σ^2_{GW} are the between- and within-population variance components of the phenotypic trait. Statistical tests and computations were conducted in R (version 3.2.2, "Fire Safety", R Core Team 2015).

Microsatellite analyses

We re-analysed the genetic data set of Macaronesian plovers previously published (Küpper *et al.* 2012) comprising in total 124 individuals. We excluded marker C204 from the published data set because this marker amplified the same locus as marker Calex-14.

We used ARLEQUIN version 3.01 (Excoffier *et al.,* 2005) to compute indices of genetic variation within and among populations including mean number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E). Pairwise F_{ST} values among populations were used to quantify the degree of population genetic differentiation, and F_{IS} statistic to estimate the inbreeding coefficient value. The Bayesian clustering software

STRUCTURE, version 2.3.4 (Pritchard *et al.,* 2000), was used to determine population structure. We run two sets of models: i) without location prior as in Küpper *et al.,* (2012) and ii) with location prior grouping samples according to archipelago or country. Using the location prior has been shown to identify meaningful genetic structure when the amount of available genetic data (samples or markers) is low (Hubisz *et al.,* 2009). The analyses aimed to assign an individual's likelihood of belonging to a certain genetic cluster (K) based on the admixture model with correlated allele frequencies (Falush *et al.,* 2003). For each approach, 15 independent simulations with K values ranging from 1 to 7 were performed for 500,000 generations with a burn-in of 50,000 generations and the five runs with the lowest Ln probability were discarded to avoid multimodality. We then assessed the assignment probabilities, logged likelihoods and, delta K (Evanno *et al.,* 2005) using STRUCTURE HARVESTER (Earl and Von Holdt, 2012) to identify the most appropriate value of K. Results of the retained ten runs for each K were summarised using CLUMPP (Jakobsson and Rosenberg, 2007) and visualised with DISTRUCT (Rosenberg, 2004).

Relationships between genetic, phenotypic and geographical distances

Geographic distance was calculated between pairs of locations, evaluated using Google Earth (http://earth.google.com). To test for the relationship between genetic, phenotypic and geographic distances, we performed Mantel tests (Mantel, 1967) using matrices of pairwise F_{ST}, P_{ST}, and geographical distances (log km). Mantel tests were performed using the package ade4 in R with simulated *P* values based on 10,000 permutations (Dray and Dufour, 2007). In addition, D_{EST} (Jost, 2008) was used to evaluate genetic differentiation between populations. This metric may provide a better assessment of differentiation compared to F_{ST} (e.g. Heller and Siegismund 2009, but see Whitlock, 2011), and we used the R package DEMEtics (Gerlach *et al*. 2010) to calculate pairwise *D* values and test whether this alternative metric of genetic distance showed a different association with geographic or morphological distances than F_{ST}. We used Bonferroni correction to calculate *P* value thresholds to account for multiple testing with the five pairwise comparisons involved (adjusted significance threshold = 0.01).

Results

Morphological differentiation

Wing and tarsus length were significantly different between populations (Figure 2). Male plovers had longer tarsi than females (males: 30.00 ± 1.3 mm [mean ± SD], females: 29.3 ± 1.4 mm) ($F_{(1,5)} = 87.71$, $P < 0.001$), although wing length did not differ between sexes (males: 109.5 ± 3.5 mm, females: 109.1± 3.4 mm) ($F_{(1,5)} = 2.86$, $P = 0.09$). Sex differences were

consistent between populations as indicated by the non-significant interaction term between sex and population (wing: F(1,5) = 0.29, P = 0.92; tarsus: F(1,5) = 1.14, P = 0.34).

Wing lengths were most similar between Fuerteventura and Santa Maria or Fuseta as indicated by the low P_{ST} values for both sexes (Table 2), whereas the least similar ones were between Oued Gharifa and Maio indicated by high P_{ST} values for both sexes (Table 2). Tarsus lengths were most similar between Oued Gharifa and Samouco or Fuseta, whereas the least similar ones were between Fuerteventura and Maio for both males and females (Table 2).

Genetic diversity and population differentiation

The lowest number of alleles was found in Madeira (2.31 ± 0.60, Porto Santo) whereas the highest were found in mainland Portugal (9.45 ± 3.85, Samouco, Table 3). No evidence of inbreeding was found in any of these populations as indicated by non-significant F_{IS} values (Table 3).

Pairwise F_{ST} comparisons between archipelagos (mean F_{ST} between archipelagos) showed significant genetic differentiation between archipelagos, and lower but still significant genetic differentiation between Boa Vista and Maio Kentish Plovers (i.e. within Cape Verde, Table 4). Pairwise *D* values demonstrated strong population structure and displayed a pattern of difference comparable to pairwise F_{ST} values for populations with $N > 2$ (Table 4).

Results from clustering analyses using STRUCTURE without location prior suggested the presence of four genetic clusters as best model splitting all archipelago populations except Madeira from the mainland population. However, when using the more sensitive method with location prior the two samples from Madeira were assigned to a separate cluster (Figure 1b). The archipelago populations were genetically distinct from the mainland population, there was only a single cluster for the two mainland populations (Iberia and North Africa), and the samples from the two Cape Verdean Islands were grouped together (Figure 1).

Genetic and morphological differentiation in relation to geographic distance

The two indices of genetic differentiation F_{ST} and D_{EST} were correlated with each other (Mantel test: r = 0.93, $P < 0.001$). Genetic differentiation estimated from microsatellites tended to correlate positively with geographical distance, however, the association was not significant after correction for multiple testing (Mantel tests: r = 0.30, $P = 0.08$; r = 0.50, $P = 0.04$, for F_{ST} and D_{EST} statistics, respectively). Similarly, there was no significant association between geographic distance and morphological differentiation, or morphological and genetic differentiation (Table 5).

Discussion

Our study found three major patterns. First we show that each archipelago is genetically differentiated, which is in line with previous studies using native bird populations in Macaronesia (Dietzen *et al*., 2003; Päckert *et al*., 2006; Illera *et al*., 2007; Rodrigues *et al*., 2014). Using a superior clustering algorithm that can deal better with low sample sizes (Hubisz *et al.,* 2009) we also found significant and meaningful genetic differences between Madeira Kentish Plovers and the mainland population despite having only sampled two unrelated birds in this archipelago. These differences had not been detected previously (Küpper *et al.* 2012). We noted during extensive fieldwork on Cape Verde, that in contrast to mainland populations, island plovers are more monogamous and highly site faithful, and tend to return year after year to breed in the vicinity of their former territory (T. Székely pers. obs.). This limited dispersal over large number of generations may have produced the genetic difference we observed between different islands.

Second, we show that for the two morphological traits analysed there are moderate differences among populations according to ANOVA and pairwise P_{ST} comparisons. Although Kentish Plovers use fairly similar habitats across their vast geographic range that includes saltpans, sand dunes, and lake shores, it is plausible that differences in microhabitat features across Macaronesia could explain the morphological differences found. Thus, perhaps, local adaptation to different ecological settings could be driving such morphological variation between archipelagos. Alternatively, wing length and tarsus length may be involved in sexual selection: as traits used by females in mate choice decisions and/or by males competing for territories and females. Males spend substantial time on courting and displaying females (Carmona et al. 2015), and they also have vicious fights with other males. Body size may well influence the outcome of these fights since agility and manoeuvrability do seem to matter in mate choice decisions in shorebirds (Szekely *et al.* 2004). Testing these alternatives would be highly rewarding and they will require assessing the direction and intensity of both natural and sexual selection on wing length and tarsus length.

Both indices of genetic differentiation (F_{ST} and D_{EST}) were correlated with each other. Neither of the indices of genetic differentiation was linked to morphological differentiation, nor was genetic or morphological differentiation significantly associated with geographic distance. For the former pattern, we propose that morphological differentiation is more likely driven by environmental conditions – that are idiosyncratic on each island – rather than by genetic

differences. However, this needs to be investigated further by using larger sample sizes to achieve higher statistical power.

In conclusion, using a widespread shorebirds species, the Kentish Plover, as a model organism we show that Macaronesian archipelagos harbour genetically and morphologically unique populations. The detected genetic and morphological differences may warrant a review of current conservation management. We suggest that each archipelago is better treated as an independent management unit based on the detected differences in microsatellite allele frequencies between archipelagos (Moritz, 1994). Such a decision will be crucial to develop urgent specific conservation actions on those populations with an increasing level of conservation concern (e.g. Canary Islands), which populations are rapidly declining.

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SUPPLEMENTARY ELECTRONIC MATERIAL

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Table S1. Result of pairwise Tukey HSD tests for wing and tarsus length of Kentish Plovers from different Macaronesian populations.

Table 1. Sampling details of Kentish Plovers used in genetic and morphometric analyses. [***Tabla 1.*** *Detalles del muestreo de Chorlitejos patinegros usados en los análisis genético y morfológico*]

Site		**Population**	**Latitude**	**Longitude**	**Morphometric (*N*)**	**Analyses**	**Genetic Analyses (*N*)**
					Male	**Female**	
Macaronesia	Cape Verde	Boa Vista (CVB)	16°04.98'N	22°54.00'W	NA	NA	11
	Cape Verde	Maio (CVM)	15°09.00'N	23°13.02'W	301	375	25
	Azores	Santa Maria (STM)	36°58.02'N	25°09.00'W	38	38	25
	Canaries	Fuerteventura (FUV)	28°43.98'N	13°55.98'W	12	14	11
	Madeira	Porto Santo (PST)	33°04.00'N	16°21.00'W	NA	NA	2
Mainland	Portugal	Samouco (SAM)	38°44.00'N	8°59.00'W	35	35	25
		Fuseta (FUS)	37°02.50'N	7°45.00'W	34	38	NA
	Morocco	Oued Gharifa (OUG)	35°30.00'N	6°24.00'W	10	11	25

NA no data available, *N* number of individuals. [NA *sin datos.* N *número de individuos*]

Table 2. Pairwise morphological differentiation (P_{ST}) for male and female Kentish Plovers for a) wing length and b) tarsus length. Males are above the diagonal, females below. Significant comparisons ($P < 0.05$) are presented in bold. [***Tabla 2.*** *Diferenciación morfológica por pares (P_{ST}) para machos y hembras de Chorlitejo patinegro en a) longitud alar y b) longitud del tarso. Los machos están sobre la diagonal, las hembras abajo. Las comparaciones significativas ($P < 0.05$) se muestran en negrita.]*

a) Wing length

Population	Maio	Santa Maria	Fuerteventura	Oued Gharifa	Samouco	Fuseta
Maio		**0.36**	**0.30**	**0.49**	**0.19**	**0.34**
Santa Maria	**0.30**		-0.03	0.06	0.04	-0.01
Fuerteventura	**0.29**	-0.02		0.12	< 0.01	-0.03
Oued Gharifa	**0.46**	0.06	0.06		**0.15**	0.06
Samouco	**0.13**	0.07	0.06	**0.24**		0.03
Fuseta	**0.27**	-0.01	-0.02	0.07	**0.04**	

b) Tarsus length

Population	Maio	Santa Maria	Fuerteventura	Oued Gharifa	Samouco	Fuseta
Maio		**0.11**	**0.69**	**0.33**	**0.40**	**0.20**
Santa Maria	< 0.01		**0.58**	0.08	**0.20**	0.01
Fuerteventura	**0.71**	**0.70**		**0.31**	**0.39**	**0.64**
Oued Gharifa	**0.20**	**0.13**	**0.66**		-0.02	0.03
Samouco	**0.35**	**0.31**	**0.54**	0.03		**0.14**
Fuseta	**0.13**	**0.07**	**0.54**	-0.02	**0.08**	

Table 3. Genetic diversity of Kentish Plovers in Macaronesia (mean ± SE). *N:* Number of individuals, N_A*:* Allele number, H_O*:* observed heterozygosity, H_E*:* expected heterozygosity. [***Tabla 3.*** *Diversidad genética de los Chorlitejos patinegro en Macaronesia* (media ± ES). N: *número de individuos,* N_A*: número de alelos,* H_O: *heterocigosidad observada,* H_E: *heterocigosidad esperada]*

Site	***N***	N_A	H_O	H_E	F_{IS} **(*P*)**
Boa Vista	11	4.75 ± 1.68	0.61 ± 0.23	0.68 ± 0.12	0.10 (0.05)
Maio	25	5.80 ± 2.38	0.65 ± 0.16	0.67 ± 0.14	0.03 (0.31)
Fuerteventura	25	8.00 ± 2.75	0.76 ± 0.16	0.77 ± 0.12	0.011 (0.33)
Oued Gharifa	11	6.35 ± 2.23	0.72 ± 0.16	0.74 ± 0.14	-0.002 (0.57)
Porto Santo	2	2.31 ± 0.60	0.75 ± 0.26	0.66 ± 0.15	-0.31 (1.00)
Samouco	25	9.45 ± 3.85	0.75 ± 0.15	0.78 ± 0.12	0.03 (0.11)
Santa Maria	25	4.75 ± 1.62	0.65 ± 0.20	0.65 ± 0.17	-0.02 (0.73)

Table 4. Pairwise F_{ST} values (above diagonal) and pairwise D_{EST} values (below diagonal) are shown. Significant comparisons (P < 0.05) are highlighted in bold. [***Tabla 4.*** *Valores de* F_{ST} *por pares (sobre la diagonal) y valores de* D_{EST} *por pares (bajo la diagonal). Las comparaciones significativas* (P < 0.05) *se muestran en negrita]*

Population	Boa Vista	Maio	Santa Maria	Fuerteventura	Porto Santo	Samouco	Oued Gharifa
Boa Vista		**0.01**	**0.18**	**0.09**	**0.25**	**0.07**	**0.11**
Maio	**0.03**		**0.18**	**0.09**	**0.24**	**0.08**	**0.11**
Santa Maria	**0.4**	**0.43**		**0.10**	**0.18**	**0.07**	**0.11**
Fuerteventura	**0.29**	**0.31**	**0.31**		**0.15**	**0.02**	**0.06**
Porto Santo	0.48	0.49	**0.33**	0.33		**0.10**	0.16
Samouco	**0.23**	**0.25**	**0.26**	**0.11**	**0.24**		**0.03**
Oued Gharifa	**0.31**	**0.31**	**0.30**	**0.23**	0.32	**0.09**	

Table 5. Partial correlations between phenotypic (P_{ST}) and geographic or genetic (F_{ST}) distance matrices are shown. P value is shown in brackets. [***Tabla 5****. Correlaciones parciales entre las matrices de distancias fenotípicas (*P_{ST}*) y geográficas o genéticas (*F_{ST}*). Los valores de significación se muestran entre paréntesis]*

Variables	**Males**		**Females**	
	Wing	**Tarsus**	**Wing**	**Tarsus**
	***r* (*P*)**	***r* (*P*)**	***r* (*P*)**	***r* (*P*)**
P_{ST} and F_{ST}	0.53 (0.12)	-0.16 (0.58)	0.21 (0.33)	-0.33 (0.73)
P_{ST} and geogr. distance	0.79 (0.07)	0.06 (0.36)	0.36 (0.23)	-0.20 (0.58)

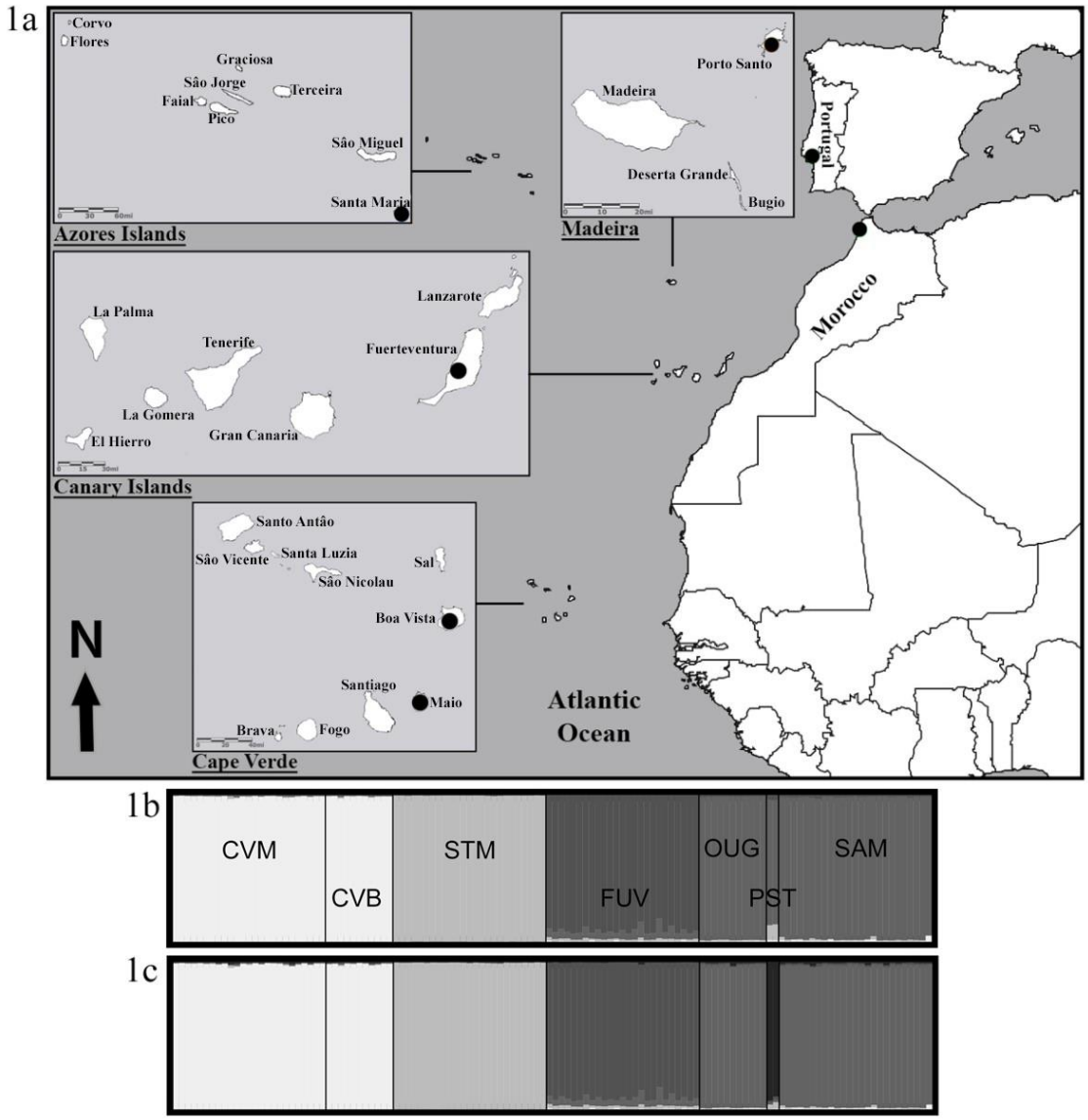

Figure 1. a Geographic locations of seven Macaronesian Kentish Plover breeding populations. **b** Assignment plot for Kentish Plovers from Macaronesia for best STRUCTURE model without location prior (K = 4). **c** Assignment plot for Kentish Plovers from Macaronesia for best STRUCTURE model with location prior (K = 5). Maio (CVM), Boa Vista (CVB), Santa Maria (STM), Fuerteventura (FUV), Oued Gharifa (OUG), Porto Santo (PST), Samouco (SAM). For colour coding of genetic clusters see map. [***Figura 1. a*** *Localización geográfica de siete poblaciones reproductoras de Chorlitejo patinegro.* ***b*** *Gráfico de asignación para los chorlitejos patinegros de la Macaronesia según el mejor modelo de STRUCTURE sin término de localización a priori (K = 4).* ***c*** *Gráfico de asignación para los chorlitejos patinegros de la Macaronesia según el mejor modelo de STRUCTURE con término de localización a priori (K = 5). Maio (CVM), Boa Vista (CVB), Santa Maria (STM), Fuerteventura (FUV), Oued Gharifa (OUG), Porto Santo (PST), Samouco (SAM)*. Para el código de colores de los grupos geneticos véase el mapa]

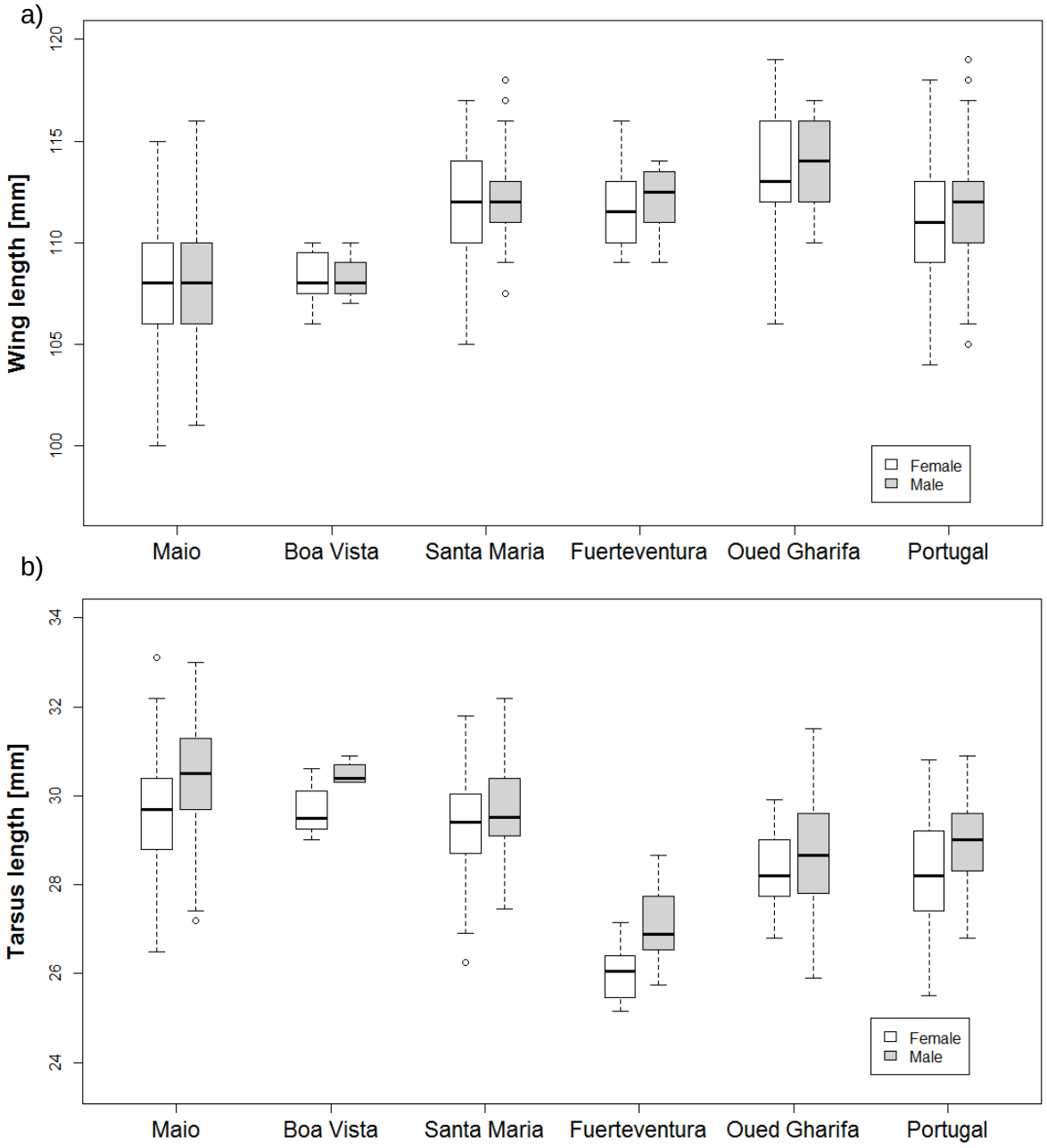

Figure 2. Wing length (a) and tarsus length (b) of male and female Kentish Plovers in Macaronesia. [***Figura 2****. Longitud alar (a) y longitud del tarso (b) de los machos y las hembras de Chorlitejo patinegro en Macaronesia*]

Appendix II
High fidelity: extra-pair fertilisations in eight *Charadrius* plover species are not associated with parental relatedness or social mating system

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This paper reports on original research I helped to conduct during the period of my Higher Degree by Research candidature.

Author contributions

KM: molecular analysis, statistical analysis, manuscript preparation
LE-P: data/sample collection in the field, manuscript preparation
AK: data/sample collection in the field, manuscript improvement
MCC-I: data/sample collection in the field, data cleaning/checking, statsadvice
MC-L: data/sample collection in the field
SZ: data/sample collection in the field
JSC: data/sample collection in the field
MA: data/sample collection in the field
MAW: data/sample collection in the field
MAS-M: data/sample collection in the field
OK:manuscript improvement
JH:manuscript preparation
TS: study conception and design, manuscript preparation
TB: manuscript preparation
CK: study conception and design, manuscript preparation

Abstract

Extra-pair paternity is a common reproductive strategy in many bird species. However, after decades of research it still remains largely unclear why extra-pair paternity occurs and why it varies among species and populations. Plovers (*Charadrius* spp.) exhibit considerable variation in reproductive behaviour and ecology, making them excellent models to investigate the evolution of social and genetic mating systems. We therefore assessed support for three major hypotheses of why extra-pair paternity occurs using a comparative approach based on the microsatellite genotypes of 2,049 individuals from 510 plover families sampled from twelve populations that constituted eight species. Extra-pair paternity rates were very low (0 to 4.1% of chicks per population). No evidence was found in support of the sexual conflict or genetic compatibility hypotheses, and there was no seasonal pattern of extra-pair paternity (EPP). The low prevalence of EPP is consistent with a number of alternative hypotheses including the parental investment hypothesis, which suggests that high contribution of males into care restricts female plovers from engaging in extra-pair copulations. Further studies are needed to critically test the importance of the parental investment hypothesis in plover mate choice.

Key words: extra-pair paternity, parental investment, breeding system

Introduction

Social and genetic mating systems are often discordant, particularly in birds (Griffith et al. 2002, Matysioková and Remeš 2013). Although 90% of bird species are socially monogamous (Lack 1968), a large proportion of species are not genetically monogamous (Griffith et al. 2002, Westneat and Stewart 2003). Despite being the focus of behavioural research for almost three decades, the reasons why females engage in extra-pair copulations (EPCs) remain unclear (Burke and Bruford 1987, Birkhead et al. 1990, Jennions and Petrie 2000, Griffith et al. 2002, Westneat and Stewart 2003, Akçay and Roughgarden 2007, Schmoll 2011, Forstmeier et al. 2014). Although a direct increase in fecundity is an immediate and obvious potential benefit to males (Petrie and Kempenaers 1998), it is still uncertain whether EPCs are an adaptive strategy of females or whether they could be the result of coercive strategies of males (Westneat and Stewart 2003, Griffith 2007, Adler 2010, Hsu et al. 2014).

It is likely that multiple factors have influenced the evolution of extra-pair paternity (EPP) and the frequency at which EPP occurs within a given population. The degree of EPP varies greatly, both intra- and inter-specifically (Petrie and Kempenaers 1998, Arnold and Owens 2002). In order for EPCs to be adaptive to females, the benefits of engaging in EPCs must outweigh the potential costs in terms of, for example, increased exposure to disease, loss of paternal care from the social mate and increased predation risk (Westneat and Stewart 2003). It has been suggested that females gain either direct or indirect benefits from engaging in EPCs. Direct benefits may include increased access to resources in return for engaging in sexual activity with a male (Gray 1997a, Petrie and Kempenaers 1998). Alternatively, EPCs could help to ensure fertility if the social partner for some reason has low fertility (Sheldon 1994, Gray 1997b). Other hypotheses suggest that females may gain indirect benefits, with offspring inheriting higher quality, “good genes” or more compatible genes, which confer heterozygote advantage for the offspring, although evidence to support this hypothesis is mixed (Petrie and Kempenaers 1998, Tregenza and Wedell 2000, Akçay and Roughgarden 2007, Schmoll 2011, Hsu et al. 2014). The genetic compatibility hypothesis suggests that extra-pair paternity occurs to reduce the deleterious effects of inbreeding (Kempenaers et al. 1999; Tregenza and Wedell 2000; Blomqvist et al. 2002a; Thuman and Griffith 2005). This hypothesis assumes that EPC-seeking females are constrained in their choice of social mates and pair with a genetically suboptimal mate. Individuals paired with more genetically similar mates therefore should engage in more EPC and experience higher frequencies of

EPP than less related pairs. Until recently, overall support for this hypothesis was considered to be weak (Akçay and Roughgarden 2007). However, a recent meta-analysis confirmed a significant positive relationship between EPP and pair relatedness (Arct et al. 2015). Inconsistencies between studies could reflect the incomparability of these studies due to methodological constraints or could be exposing true differences among genera, species or populations. Multi-species studies, where both field and laboratory work has been conducted following consistent protocols, eliminate such methodological heterogeneity and permit more reliable and robust tests of this and other hypotheses proposed to explain EPPs.

Sexual conflict could also be a driver of EPP within a species or population (Westneat and Stewart 2003, Székely et al. 2007). Conflict between mates occurs as a result of contrasting evolutionary interests of the sexes over reproduction at both pre-and post-zygotic levels (Parker et al. 2002, Thomas et al. 2007). For example, conflict can develop from opposing optimal mating and fertilisation or parental care strategies (Székely et al. 2007, Thomas et al. 2007). Interactions between sexual selection, mating opportunities and parental care cause an evolutionary feedback loop between mating systems and parental care behaviours (Thomas et al. 2007). Brood desertion for remating by one of the parents is an extreme resolution of this conflict. The deserting parent gains increased reproductive success by pairing with a new mate, whereas the abandoned mate must bear the full cost of raising the brood alone. Sexual conflict theory predicts that extra-pair young (EPY) will be more frequent in socially polygamous species compared to those that are socially monogamous and experience lower levels of conflict.

The genus *Charadrius* consists of 30 species of plovers belonging to the order Charadriiformes, which breed on every continent except Antarctica (Dos Remedios et al. 2015). The social mating systems and parental care strategies of plovers vary greatly (Székely et al. 2006, 2007). Breeding systems vary from biparental care and monogamy to uniparental care and polygamy – where one parent deserts the brood soon after hatching (Székely and Cuthill 1999, Kosztolányi et al. 2006). This diversity in mating systems and ecology within a single genus make plovers excellent study species in which to investigate the evolution of breeding systems (Vincze et al. 2013). Despite good knowledge of social mating systems in plovers, little is known about their genetic mating systems (Küpper et al. 2004).

The occurrence of EPP within a population sometimes follows a seasonal pattern (Dale et al. 1999, Küpper et al. 2004). One explanation is that the availability of high

quality mates late in the breeding season is limited to females since most high quality males will already be paired up, meaning that they often end up pairing with a low quality mate. At the same time chick mortality often increases with season as environmental conditions deteriorate and competition between families increases (Székely and Cuthill 1999, Székely et al. 1999, Küpper et al. 2004, Kosztolányi et al. 2006, 2009). Females might then be more likely to engage in extra-pair copulations to obtain genetic benefits for their offspring and increase offspring survival. Alternatively, higher rates of EPP in late clutches could be a result of sperm storage in sequentially polyandrous females (Dale et al. 1999, Oring et al. 1992; Küpper et al. 2004).

Here, we used parentage assignment based on microsatellite genotyping of 510 families and 1,071 offspring sampled from twelve populations of eight species to investigate the degree of concordance between plover social and genetic mating systems. Specifically, we tested whether the frequency of EPP was related to the social mating system as predicted by sexual conflict theory. We predicted that socially polygamous populations experiencing higher levels of conflict would have higher frequencies of EPP than more cooperative, socially monogamous populations. Second, we tested the genetic compatibility hypothesis using our multi-population data set. Plovers are appropriate models for investigating the genetic compatibility hypothesis as previous work suggested that this may be an important driver of EPP frequency in shorebirds (Blomqvist et al. 2002a; Thuman and Griffith 2005). We predicted that social pairs with higher genetic relatedness would be more likely to have EPY than more distantly related individuals. Finally, we tested whether the incidence of EPP was related to the timing of mating within the breeding season. We predicted that the frequency of EPP would be higher at the end of the breeding season to compensate for constrained mate choice and more challenging conditions for offspring survival.

Materials and Methods

Sample collection and DNA extraction

We collected samples from twelve populations constituting eight *Charadrius* species. These populations included five populations of Kentish plover *C. alexandrinus* (KeP1-5 from the Azores, Cape Verde, Turkey, United Arab Emirates and Saudi Arabia) and one population each of two-banded plover *C. falklandicus* and rufous-chested dotterel *C. modestus* (TbP and RcD from Falklands Islands), white-fronted

C. marginatus, Kittlitz's *C. pecuarius*, Madagascar plover *C. thoracicus* (WfP, KiP, and MaP from Madagascar), snowy plover *C. nivosus* (SnP, Mexico), and red-capped plover *C. ruficapillus* (RcP, south-eastern Australia). KeP2 was also analysed by Blomqvist et al. (2002a). The plover populations vary both in breeding systems and parental care strategies (Table 1).

Details regarding fieldwork and specific conditions for each population are described elsewhere (see references in Table 1). In brief our general methodology across all populations: we searched for nests on foot or by car according to standardised field methods outlined in Székely et al. (2008). Adults were caught on the nest during incubation or while tending newly-hatched chicks using funnel traps. The majority of chicks were caught shortly after hatching in or around (i.e. < 20 m) the known nest scrape. 25–50 µl of blood was collected for DNA extractions from the brachial or tarsal vein in adults or tarsal vein in chicks. Blood samples were stored either in Queen's Lysis Buffer (Seutin et al. 1991) or absolute ethanol until extraction. Parents and chicks were colour ringed or flagged to allow assignment of social parents during subsequent encounters of families in the field. All samples were collected between 1998 and 2014 (Table 1).

We extracted DNA using the ammonium acetate precipitation method (Nicholls et al. 2000). DNA quality was evaluated by visualising DNA on a 0.8% agarose gel stained with SYBRsafe (Invitrogen, Carlsbad, California) or ethidium bromide, and we assessed the quantity of DNA using a NanodropND800 (Thermo Fisher Scientific).

Genotyping

We used microsatellite markers to obtain genetic profiles for each sampled bird and assign parentage. We combined fluorescently labelled primer pairs into multiplexes and typed 11–26 microsatellite markers per species (Table S1) plus 1–3 sexing primers (Griffiths et al. 1998, Küpper et al. 2007). Kentish plover multiplexes designed by Küpper et al. (2009) were used to genotype Kentish, white-fronted and red-capped plovers. For the other five species, we tested marker sets from related species for cross-amplification and markers with proven utility in other bird species (Primmer et al. 1995, Funk et al. 2007, Küpper et al. 2007, 2008, Dawson et al. 2010, 2013). PCR reactions were run using 2 µl Qiagen multiplex mix (1 µl Qiagen master mix to 1 µl primer mix) with 1–15 ng DNA. We undertook PCRs using a programme of one cycle of 15 min at 95°C followed by 35 cycles at 94°C for 30 s, Ta (multiplex specific annealing temperature, Table S1) for 90 s, 72°C for 60 s and a final extension cycle 60°C for 30 min, in a thermal cycler (MJ Research TETRAD2 DNA

Engine). A small fraction of the PCR product was loaded onto an ABI 3730 DNA fragment analyzer. We assigned allele sizes to allele bins using GeneMapper v3.7 software (Applied Biosystems). Individuals that were typed at fewer than 80% of the markers were excluded from further analysis. Using the adults only, we calculated frequency of null allele and a combined non-exclusion probability of all markers for each population using the software Cervus 3.0.3 (Kalinowski et al. 2007).

Parentage analysis

The number of families studied per population varied from 10 to 100 (Table 2). Our parentage assignment procedure had two steps. Firstly, we conducted parentage assignment using the social parents as sole candidate parents in Cervus 3.0.3 (Kalinowski et al. 2007). Cervus works by assessing the parentage likelihood of the candidate parent for a given offspring and provides the confidence of the parentage assignment relative to the critical LOD or Delta scores, which is generated through simulation. Simulation parameters were set as follows: 10,000 cycles, 90% candidate parents sampled, 5% loci mistyped (null alleles or genotype mismatch) and population-specific numbers for loci and candidate parents (Table 1). Although theory suggests that the parent and offspring should match genotypes completely, mismatches can occur due to mutations or genotyping error (Hoffman and Amos 2005). Cervus uses a probability-based approach to account for those mismatches, which occasionally can lead to unlikely results considering other sources of knowledge from the parents (Bouwman et al. 2006). Therefore, we reviewed the relationship between social parents and chicks and determined the number and nature of mismatches. We accepted social parents as genetic ones if they and their putative chicks had no more than one true mismatch and one null allele mismatch. We checked all incidences of two or more allele mismatches in detail. Mismatches caused by null alleles are a particular problem when cross-amplifying markers are used, since the match between primer and target sequences will be poorer than in the species in which the marker was developed. Consequently, null allele frequencies increase with increasing phylogenetic distance from the target species (Li et al. 2003), which could potentially lead to the false exclusion of true parents (Dakin and Avise 2004). On the other hand, dropping all markers affected by null alleles severely reduced exclusion probabilities in some populations. Therefore, we only dropped markers affected by null alleles for a given species until the combined non-exclusion probability score had dropped below 0.95 (see Table S2 for non-exclusion probabilities). In these cases, (i.e. rufous-chested dotterel, Kittlitz's, white-fronted, Madagascar and snowy plover), we only rejected parents if parent-offspring

dyads showed at least three mismatches, including one 'true' mismatch that could not be explained by null alleles. Instances where both parents did not match the chick genotype were classified as cases of intra-specific brood parasitism (IBP) and cases where only the female was excluded were classified as quasi-parasitism (QP). IBP can occur when a female lays an egg in another pairs' nest, whereas QP occurs when a female lays an egg in the nest after copulating with the paired male first (Yom-Tov 2001, Griffith et al. 2004, Lyon and Eadie 2008, Berger et al. 2014).

Mismatches between parents and offspring can potentially result from incorrect assignments of parents during laboratory or fieldwork due to human errors. In families where chicks did not match the parents, all individuals were independently re-extracted and re-genotyped to ensure errors during laboratory work were not responsible for the observed mismatches. For all mismatched families remaining in the relaxed dataset (n = 26), we also excluded other error sources, for example, by confirming the sex of putative parents using molecular markers (n = 9) to ensure that always a male and a female had been assigned as social parents during fieldwork.

Potential sources of parental mis-assignments introduced during fieldwork by sampling the wrong precocial chicks are given in Table 3. Since we were only interested in the frequencies of EPY that resulted from alternative mating behaviour, we created two datasets: (i) a high-stringency dataset ('strict' dataset), which includes only broods with both parents sampled or identified during incubation before the last egg had hatched; and (ii) a low-stringency data set ('relaxed' dataset), including additional families that originated from an undiscovered nest, or for which the chicks (or parents) were sampled after they had left the nest scrape. In addition, we also thoroughly checked field notes for all families where the social parents had been rejected to identify and eliminate further sources of potential error.

Social vs genetic mating systems

We tested whether the social breeding system was related to the degree of EPP using Fisher's exact test. For this analysis, populations were counted as either having an incidence of EPP/extra-pair fertilisation (EPF, where either the male or female were excluded as the genetic parent) or no EPP/EPF. Six populations were included where information was available for both mating system and parental care strategies. We defined a population's breeding system using a combination of their mating system and parental care strategy. Populations were classified as polygamous (snowy plover, Kittlitz's plover and Kentish plover from Turkey) if they exhibit high levels of within season sequential polygamy (>5%; Dunn et al. 2001;

Eberhart-Phillips unpublished data) and also uniparental care (Table 1). Monogamous populations (Madagascar plover, white-fronted plover and Kentish plover from Cape Verde) have high levels of between season monogamy (Eberhart-Phillips unpublished data) and biparental care (Table 1). It is important to note however, that variation in mating and parental care strategy does occur within all populations.

Pair relatedness and temporal patterns

We used EPP/EPF presence or absence within a brood as the dependent variable and fitted population nested within species as a random factor in a series of Generalized Linear Mixed Models (GLMMs). EPF was only tested in the relaxed data set, as there were no incidences of IBP within the strict dataset. We calculated pair relatedness, i.e. the relatedness between the social father and mother, using Queller and Goodnight's r (Queller and Goodnight 1989) in the program SPAGeDi 1.5 (Hardy and Vekemans 2002) to test whether parental relatedness predicted the occurrence of EPFs. To test for a temporal pattern of EPP, we tested whether the occurrence of EPFs over the breeding season was different from a random distribution or not using a GLMM. For each population, we standardised hatching date based on the available data by subtracting the mean and dividing by the standard deviation of each population. We also tested for an interaction between pair relatedness and standardised hatching date. These analyses were only carried out on populations and nests where hatching date information was available, Kentish plover from the Azores, two-banded plover and rufous-chested dotterel were excluded from both the restricted and relaxed dataset analysis, whereas red-capped plover was excluded from the strict dataset analysis only. We fitted a GLMM with a binomial error structure and a cloglog link function to the data. The cloglog link function allows for more asymmetry in the distribution than the standard logit link function. The GLMM was also fitted to the entire strict dataset, including parents of families where hatching date information was not known, to investigate the effect of relatedness alone to confirm results of the reduced standardised hatch dataset. From saturated models that included all independent variables, relatedness and hatching date, and one two-way interaction, we sequentially removed non-significant interactions and variables until the minimal model was reached. We tested the significance of the increase in deviance as a result of model simplification using chi-square tests.

We included only one randomly chosen record of parents with multiple broods in the dataset to avoid pseudoreplication. For the correlates of EPFs, we report the results

of the strict dataset plus the two-banded plover for which we did not detect any EPFs (see Results); however we note that the results were qualitatively the same for the relaxed dataset. We used R version 3.2.3 (R Core Team 2015) for all statistical analyses.

Results

Parentage analysis and social vs genetic mating systems

A total of 1,071 offspring and 978 adults constituting 510 families from twelve populations were genotyped at up to 21 microsatellites. The frequency of EPP was rare (< 5%) within each population. All of the offspring were included in the relaxed dataset and 711 (66%) offspring from 340 (67%) families were retained in the strict dataset. Analysis of the strict dataset yielded EPP rate estimates between zero and 4.1% of broods (Figure 1; Table 2). There was no incidence of QP (Figure 1; strict dataset, Table 2). The rate of IBP was estimated at between zero and 8.3% of broods (Figure 1; strict dataset, Table 2). Analysis of the relaxed datasets yielded somewhat higher EPP, IBP and QP estimates in most populations (relaxed dataset, Table 2). Monogamous and polyandrous populations did not differ in their occurrence of EPFs or EPPs (EPF/EPP occurrence: Fisher exact test: $p = 1$).

Pair relatedness and temporal patterns

Pair relatedness of parents was low across all populations except for the Madagascar plover (Figure 2). Pair relatedness or the interaction with standardised hatching date had no influence on the occurrence of EPPs and hence was not retained in the minimal model (GLMM; interaction: $\chi2 = 0.0061$, $df = 1$, $p = 0.94$, pair relatedness: $\chi2 = 0.37$, $df = 1$, $p = 0.54$). Interestingly, the single case of IBP that occurred in the Madagascar plover occurred in a family where both parents were very closely related ($r = 0.514$). The occurrence of EPP was not significantly associated with time of the breeding season (GLMM: $\chi2 = 3.81$, $df = 1$, $p = 0.051$). However, there was a non-significant trend for EPPs to be more frequent during the latter part of the breeding season (estimate = 0.99, ± 0.94 [SE], $Z = 1.476$, $p = 0.14$), which was not apparent in the relaxed dataset. When the larger strict dataset was used to examine the effects of relatedness alone, which included previously excluded parents of families where hatching date information was not known, relatedness was still not found to influence the occurrence of EPP (GLMM: $\chi2 = 0.40$, $df = 1$, $p = 0.53$; Figure 2).

Discussion

Social and genetic mating system of plover populations

Our study found low rates of EPP and EPF across all 12 populations. Trends found during analysis were consistent between the two datasets. Previous work in shorebirds also found low levels of EPP in other *Charadrius* species: ringed plover *C. hiaticula* (0% EPP, Wallander et al. 2001), semipalmated plover *C. semipalmatus* (4.2% EPP, Zharikov and Nol 2000) and Eurasian dotterel *C. morinellus* (9.1% EPP, Owens et al. 1995), suggesting this is a widespread trend among plover species. This contrasts with high frequencies of EPP found in other bird clades, with the highest rates found in the superb fairy-wren *Malurus cyaneus*, for example, where up to 76% of offspring are sired by an extra-pair male (Mulder et al. 1994).

Taken together, our results suggest that EPP is a relatively rare strategy in plovers with limited importance. Low EPP rates could result from mate guarding and social constraints. Many plover species only produce a single clutch of three or four eggs, suggesting that the cost of losing paternity may be high for the male (Wallander et al. 2001). Behaviours that reduce loss of paternity should therefore be under strong selection in males (Westneat and Stewart 2003, Thomas et al. 2007). Mate guarding occurs in some but not all plover species (Zharikov and Nol 2000, Wallander et al. 2001). Plover breeding habitat is often open with little cover (Muir and Colwell 2010), making it difficult for females to engage in EPCs without observation or disruption from the social mate (Delehanty et al. 1998, Zharikov and Nol 2000, Wallander et al. 2001, Küpper et al. 2004). It is possible that rare cases of EPP could be gained through EPCs obtained in the evening or early morning. Indeed there is increasing evidence in species with high EPP rates that females obtain EPCs before dawn (Double and Cockburn 2000, Ward et al. 2014).

Our results hint at the possibility that plovers could be using paternity insurance strategies, such as high rates of within-pair copulations which act as paternity assurance for the social male (Emlen et al. 1998, Schamel et al. 2004). Other methods of paternity assurance may also occur, for example polyandrous wattled jacana *Jacana jacana* males have been observed to discard the first egg laid in the clutch except in the first breeding attempt of the season (Chen et al. 2008).

Alternatively, females might be constrained by their reliance on male cooperation during parental care, preventing them from seeking EPCs (Wallander et al. 2001;

Blomqvist et al. 2002b; Arnqvist and Kirkpatrick 2005). Male parental care is important in many shorebird species and the costs of reduced parental care can be high when the remaining parent is unable to compensate fully (Székely and Williams 1995; Székely and Cuthill 1999; Blomqvist et al. 2002b; Thomas et al. 2007). Biparental care is particularly important for plovers breeding in harsh environments (Kosztolányi et al. 2009, Vincze et al. 2013). Therefore, females should avoid EPCs if they result in a reduction in care, desertion or divorce from their social mate. Lack of pronounced sexual size dimorphism in plovers may better allow females resist enforced copulations by males. Shorebird females have been observed to resist extra-pair copulations and act aggressively to males other than their social mate (Lanctot et al. 2000). This suggests that at least in some shorebird species that females are not actively seeking extra-pair copulations and may actually be avoiding them.

We found that the genetic mating system did reflect the social breeding system in our study as the level of EPP was low in both monogamous and polyandrous plovers. There was no significant difference in the occurrence of EPP among different social breeding systems. For example, EPP was consistently rare across populations of the Kentish plover, despite these populations having different social breeding systems. However, because of the generally low frequencies of EPP and the moderate sample sizes, our statistical tests may have had limited power to pick up any true differences between breeding systems. Thomas et al. (2007) found that among socially monogamous shorebirds, those expressing higher levels of cooperation had lower levels of EPP. Extra-pair offspring are often more common in species experiencing higher levels of social conflict, such as polygynous and polyandrous species (Oring et al. 1992, Lanctot et al. 1997, Emlen et al. 1998, Lank et al. 2002, Székely et al. 2007, Thomas et al. 2007). This possible association was not evident in our analysis of twelve plover populations. Both monogamous and polygamous populations of Kentish plover had less than 5% EPP. Therefore, we have no evidence to suggest that sexual conflict is causing differences in EPP rates in plovers we found. A similar pattern of low EPP in polyandrous species has also been observed in several other shorebird species (Owens et al. 1995, Delehanty et al. 1998, Dale et al. 1999, Schamel et al. 2004). This lack of association can perhaps be explained by the observation that polygamous plovers are typically serially polyandrous and exhibit partial biparental care, during incubation, but not during brooding. The low frequency of EPP found in these polyandrous species perhaps reflects the importance of paternal care. This is supported by previous work which found relatively low rates of

EPP in the Eurasian dotterel, a species that exhibits sex-role reversal (i.e., females being the more showy competitive sex and males providing nearly all parental care; Owens et al. 1995).

Pair relatedness

We found no evidence that parental relatedness was correlated with the likelihood of extra-pair young. As the relatedness of the parents was low across all populations except for the Madagascar plover, inbreeding risk is probably low and thus EPP is an unnecessary strategy. These results are contrary to previous studies of shorebird species, which found that more related pairs were more likely to have extra-pair chicks (Blomqvist et al. 2002a; Küpper et al. 2004). Somewhat surprisingly, we did not find this relationship across (nor within) plover populations despite using a large number of highly polymorphic microsatellite markers (Table S1). It is possible that the difference observed between the previous work and this study could be due to methodological differences, as DNA fingerprinting was used in the earlier study of Kentish plover. Microsatellite analysis, as used here, is considered to be more accurate than DNA fingerprinting when judging genetic similarity between individuals (Reeve et al. 1992, Griffith and Montgomerie 2003). However, meta-analyses by Arct et al. (2015) found that significant positive effect sizes of EPP and relatedness were associated only when microsatellite markers were used and not other methods. This is the opposite of our findings when comparing results between studies on the same species. Thus our work highlights the importance of using consistent methodology, both in the field and laboratory with stringent filtering according to data quality, which allows for more reliable inference.

Temporal patterns

There was a non-significant trend for EPPs to be more frequent later in the breeding season. This matches previous findings in a single population of Kentish plovers (Küpper et al. 2004) and therefore is consistent with the predictions of an adaptive strategy when females are paired up with inferior mates. However, since the temporal trend was relatively weak and EPPs in general were rare, this strategy may have limited importance for plovers.

QP, IBP and EPP

Evidence for QP and IBP has been found in shorebirds and other bird species (Yom-Tov 2001; Blomqvist et al. 2002a; Arnold and Owens 2002; Griffith et al. 2004; Küpper et al. 2004; Krakauer 2008; Du and Lu 2010). IBP occurs when a female lays an egg in a conspecific pair's nest (Yom-Tov 2001, Berger et al. 2014). This can be

detrimental to the fitness of the male and female recipients, as they are forced to provide care for the parasitic chick (Petrie and Møller 1991). QP occurs when a female engages in copulations with an already-paired male and then lays an egg in the paired female's nest, so that the social female incurs the fitness cost of raising the extra-pair offspring (Griffith et al. 2004, Lyon and Eadie 2008). It has been suggested that both IBP and QP occur when a female engages in EPCs with a male and then dumps an egg, sometimes in the same male's nest (Küpper et al. 2004). The rare occurrence of IBP and absence of QP within the strict dataset suggests that these are not major strategies in plovers. It is important to note that cases of EPP and QP were much more common in the relaxed dataset, although in general the trends we found were consistent between the two datasets. Differences in the frequency of EPP, QP and IBP between the relaxed and strict datasets could reflect inaccuracies in field assignment. This highlights the benefit of large datasets, as firm conclusions can still be drawn from a subset of the data, and also emphasises the need to have stringent field protocols. It is also important to consider that the level of EPP, QP and IBP reported are conservative estimates as the capacity to detect extra-pair offspring was limited in some species due to relatively low exclusion probabilities.

There have been several hypothesis proposed to explain variation in the level of EPP. Using 12 populations of plover and stringent data filtering, no significant association was found between social breeding system, pair relatedness and temporal patterns and EPP in plovers. We also found that levels of EPP were consistently low across all of the studied populations. This is consistent with efficient mate guarding and/or a constraint on EPCs engagement by females through the substantial share of parental care that males provide. Further work is needed to critically test these hypotheses. It would be particularly interesting to test whether polygynous plover populations, in which females provide the majority of the parental care, have higher levels of EPP.

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Table 1. Study populations, social mating system, study years, parental care strategy and population information for the 12 plover populations.

Species	Population	Years	Parental care strategy	Reference
Polygamy				
Snowy plover	Mexico	2006-2010	Male only/biparental	Argüelles-Ticó 2011
Kittlitz's plover	Madagascar	2006-2013	Male only	Parra et al. 2014
Kentish plover	Turkey	1998-1999	Male only/biparental	Argüelles-Ticó 2011
Monogamy				
Kentish plover	Cape Verde	2007-2013	Biparental care	Argüelles-Ticó 2011
White-fronted plover	Madagascar	2006-2013	Biparental/male only	Parra et al. 2014
Madagascar plover	Madagascar	2009-2013	Biparental care	Zefania et al. 2008
Unknown				
Kentish plover	UAE	2005-2006	Biparental care	Argüelles-Ticó 2011
Kentish plover	Azores	2009-2012	Likely biparental care	
Kentish plover	Saudi Arabia	2011	Biparental care	Argüelles-Ticó 2011
Two-banded plover	Falklands Islands	2005-2008	Biparental care	St Clair et al. 2010a; T. Székely, unpublished data
Rufous-chested dotterel	Falklands Islands	2005-2008	Biparental care	St Clair et al. 2010b; T. Székely, unpublished data
Red-capped plover	Australia	2010-2014	Biparental care	Ekanayake et al. 2015

Table 2. The occurrence of extra-pair paternity (EPP), quasi-parasitism (QP) and intraspecific brood parasitism (IBP) across twelve plover populations. The strict data set includes only families for which candidate parents were determined unanimously in the field and therefore provides a minimal estimate of the occurrence of the alternative mating strategies. By contrast, the relaxed values provide initial parentage assessment including mis-assignments that are the likely results of wrongly assigned parentage in the field. First line in each population provides the summary of broods, the second line in italicised font provides the summary of chicks.

		Strict				**Relaxed**			
Species	**Population**	**N broods** ***chicks***	**EPP % (*n*)**	**QP% (*n*)**	**IBP% (*n*)**	**N broods** ***chicks***	**EPP % (*n*)**	**QP% (*n*)**	**IBP% (*n*)**
Snowy plover	Mexico	90 *194*	0	0	0	100 *225*	0	0	0
Kittlitz's plover	Madagascar	15 *18*	0	0	0	31 *44*	6.3 (2) *4.3 (2)*	3.1 (1) *2.2 (1)*	6.3 (2) *4.3 (2)*
Kentish plover	Turkey	73 *184*	4.1 (3) *1.6 (3)*	0	1.4 (1) *0.5 (1)*	94 *249*	4.3 (4) *1.6 (4)*	0	1.1 (1) *0.4 (1)*
Kentish plover	Cape Verde	63 *120*	1.6 (1) *0.8 (1)*	0	3.2 (2) *1.7 (2)*	90 *174*	2.2 (2) *1.7 (3)*	0	3.3 (3) *1.7 (3)*
Kentish plover	UAE	28 *57*	0	0	0	48 *101*	4.2 (2) *2.0 (2)*	0	2.1 (1) *2.0 (2)*

Kentish plover	Azores	0	-	-	-	19 *46*	0	0	0
Kentish plover	Saudi Arabia	12 *19*	0	0	0	27 *54*	3.7 (1) *1.9 (1)*	0	0
Two-banded plover	Falklands Islands	6 *15*	0	0	0	10 *26*	0	0	0
Rufous-chested dotterel	Falklands Islands	8 *14*	0	0	0	12 *19*	0	0	0
White-fronted plover	Madagascar	10 *17*	0	0	0	29 *49*	0	3.45 (1) *4.08 (2)*	3.45 (1) *2.04 (1)*
Madagascar plover	Madagascar	12 *20*	0	0	8.3 (1) *5.0 (1)*	28 *46*	0	0	3.6 (1) *2.2 (1)*
Red-capped plover	Australia	4 *7*	0	0	0	22 *38*	9.1 (2) *7.9 (3)*	13.6 (3) *10.5 (4)*	4.5 (1) *2.6 (1)*

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Table 3. Potential sources of errors for parentage assignment in the field in precocial birds, identified and addressed by this study.

Reason for mismatch	**Consequence**	**Action**
Brood mixing	Apparent case of IBP. May occur when offspring were first marked and sampled after they had left the nest scrape.	Excluded from strict data set.
Parents not caught on nest during incubation	Apparent case of EPP, QP or IBP. Occasionally adult plovers that are not the true parents are caught with chicks and assigned as candidate parents in the field.	Excluded from strict data set, if no further family re-sightings with the candidate parents exist from later dates.
Other errors during parentage assignment in the field	Apparent case of EPP, QP or IBP.	Excluded from strict data set, if field notes during capture suggested that initial assignment was uncertain.

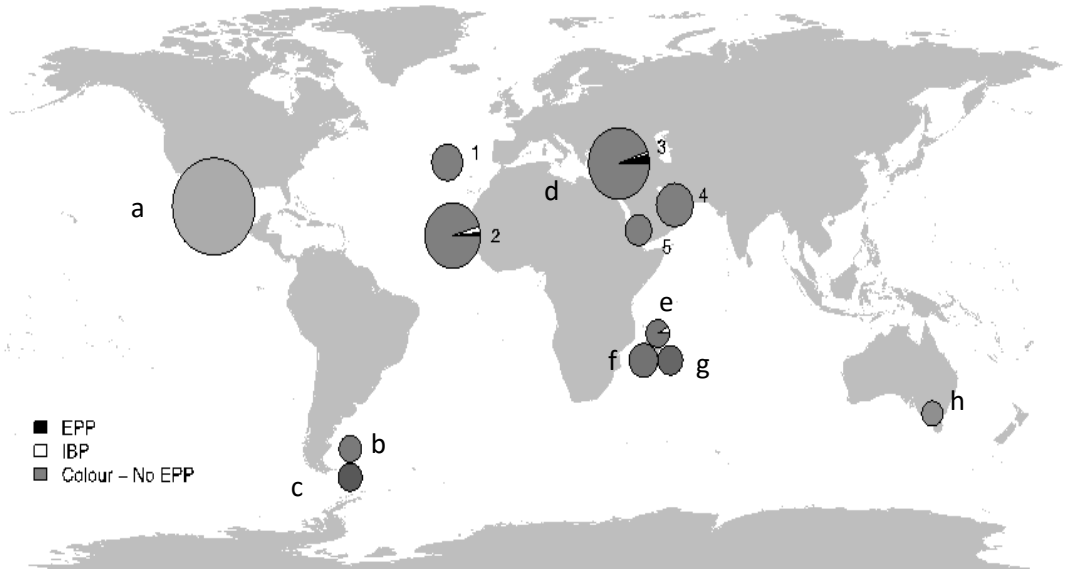

Figure 1: Frequency of extra-pair paternity (EPP) and intraspecific brood parasitism (IBP) in *Charadrius* plovers based on the strict dataset (see Tables 2 and 3). Snowy plover (a), two-banded plover (b), rufous-chested dotterel (c), Kentish plover (d: 1, Azores; 2, Cape Verde; 3, Turkey; 4, United Arab Emirates; 5, Saudi Arabia), Madagascar plover (e), Kittlitz's plover (f), white-fronted plover (g) and red-capped plover (h). Sample sizes of broods are indicated by the size of population specific pie-charts.

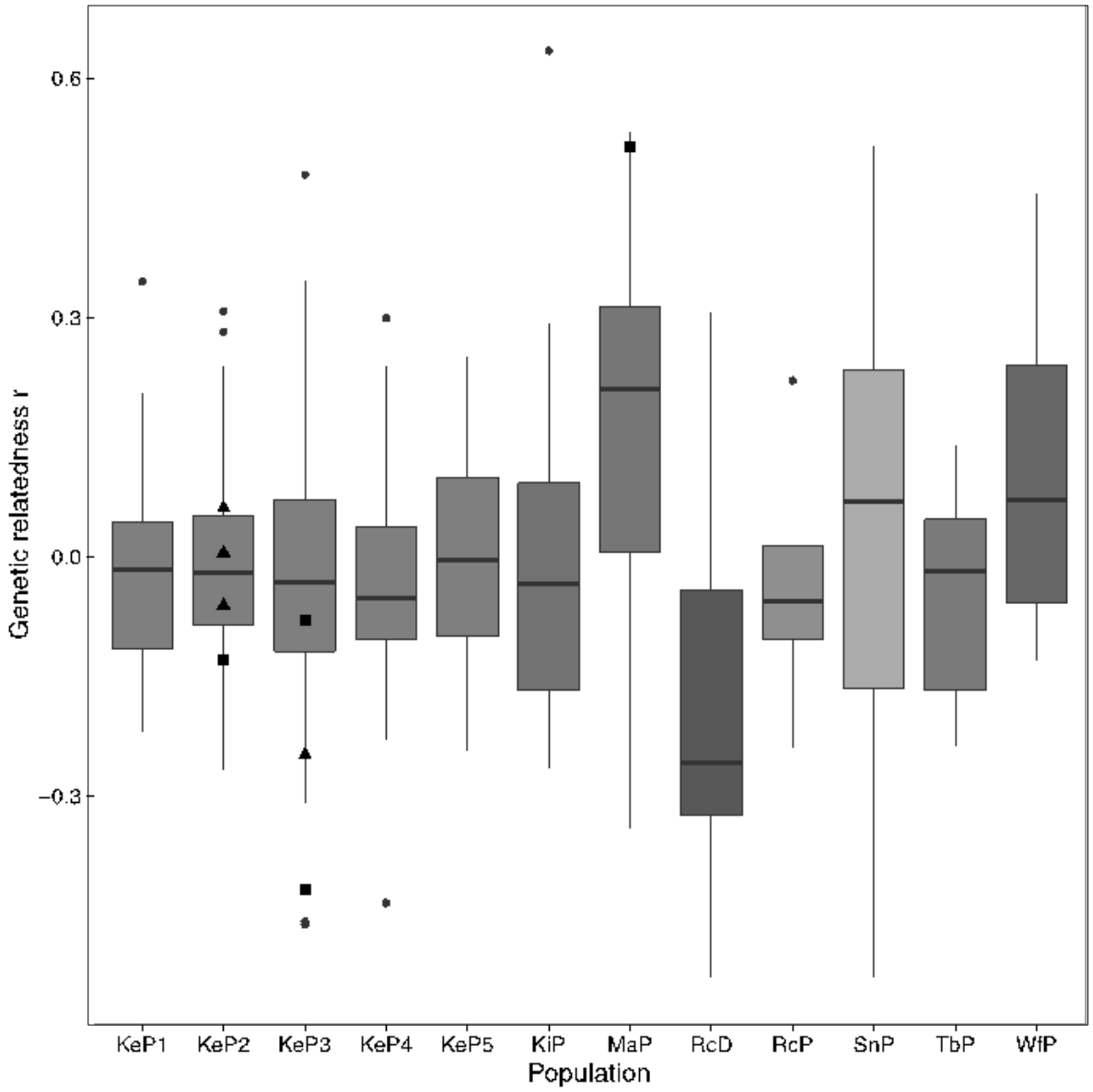

Figure 2: Relatedness (Queller and Goodnight's *r*) between social parents and the occurrence of extra-pair fertilisations across twelve plover populations. The relatedness of pairs where extra-pair paternity occurred is represented by black triangles and where intraspecific brood parasitism occurred is represented with black squares. KeP1: Kentish plover, Azores, KeP2: Kentish plover, Cape Verde, KeP3: Kentish plover, Turkey, KeP4: United Arab Emirates, KeP5: Kentish plover, Saudi Arabia, KiP: Kittlitz's plover, MaP: Madagascar plover, RcD: rufous-chested dotterel, RcP: red-capped plover, SnP: snowy plover, TbP: two-banded plover, WfP: white-fronted plover.

Table S1. Multiplex design and multiplex specific annealing temperatures (T_a) in *Charadrius* plovers.

* Primers were run using a different annealing temperature and then combined within a single multiplex post PCR.

Species	Multiplex	T_a (^oC)	Locus	Dye
Snowy plover	SP1	57	Calex02	PET
			Calex04	NED
			Calex05	FAM
			Calex10	FAM
			Calex18	FAM
			Calex19	VIC
			Calex23	PET
			Calex39	VIC
			Calex43	FAM
			CAM18	FAM
			P2/P8	NED
	SP2	60	Calex12	FAM
			Calex14	VIC
			Calex31	NED
			Calex32	FAM
			Calex35	FAM
			HrU2	NED
Kittlitz's plover	KiP1	56	Calex01	FAM
			Calex18	NED
			Calex19	NED
			Calex33	HEX
			Calex45	HEX
			BmaTATC	FAM
		*65	*Calex16	NED
	KiP2	56	Calex36	HEX
			GgaMopl15	FAM
			P2/P8	HEX
		*48	*HrU2	NED
	KiP3	56	Calex15	FAM
			Tgu06	HEX
Kentish plover, (White-fronted plover, Red-capped plover)	KP1	57	Calex02	PET
			Calex04	NED
			Calex05	FAM
			Calex08	VIC
			Calex18	NED
			Calex19	VIC
			Calex23	PET
			Calex24	FAM
			Calex39	VIC
			Calex43	FAM
			Calex45	FAM
			P2/P8	NED
	KP2	62	Calex01	FAM
			Calex11	NED
			Calex12	FAM
			Calex14	VIC
			Calex22	VIC
			Calex26	VIC
			Calex28	NED
			Calex31	NED
			Calex37	PET
	KP3	60	Calex10	HEX
			Calex32	FAM
			Calex35	HEX
			C201	FAM
			C203	NED
	KP4	60	C204	FAM
			C205	HEX

			HrU2	NED
Two-banded plover	TbP1	57	Calex02	PET
			Calex05	FAM
			Calex14	VIC
			Calex17	FAM
			Calex39	VIC
	TbP2	56	GgaMan13	HEX
			Tgu04_004	HEX
			C201	FAM
	TbP3	57	Calex07	FAM
			BmaTATC444	HEX
			Tgu06	HEX
	TbP4	62	Calex33	HEX
			Calex40	FAM
			P2/P8	NED
Rufous-chested dotterel	RcD1	56	Calex35	HEX
			Tgu01_40	FAM
			Tgu03_98	HEX
			GgaMopl21	FAM
			GgaMan13	HEX
		*48	*HrU2	NED
		*65	*Calex16	FAM
	RcD2	56	Calex11	NED
			Calex45	FAM
			Tgu06	HEX
			GgaMopl15	FAM
	RcD3	56	Tgu04_004	HEX
			GgaGmeB2	FAM
			P2/P8	NED
Madagascar plover	MP1	56	Calex01	FAM
			Calex07	FAM
			Calex10	HEX
			Calex18	NED
			Calex19	NED
			Calex45	HEX
			Tgu04_004	HEX
			Mopl6	FAM
	MP2	56	Calex11	NED
			Cakex35	HEX
			Tgu05_053	FAM
			P2/P8	NED

Table S2: Combined non-exclusion probabilities for plover populations, first parent, second parent and parent pair. Calculated in Cervus 3.0.3

Species	**Population**	**Combined non-exclusion probability (first parent)**	**Combined non-exclusion probability (second parent):**	**Combined non-exclusion probability (parent pair):**
Snowy plover	Mexico	0.11702965	0.01373164	0.00073837
Kittlitz's plover	Madagascar	0.01229723	0.0004182	0.00000222
Kentish plover	Turkey	0.00000246	1.69E-09	1.09E-15
Kentish plover	Cape Verde	0.00079275	0.00000305	3.76E-10
Kentish plover	UAE	0.00002114	0.00000004	2.10E-13
Kentish plover	Azores	0.00351729	0.00004458	0.00000005
Kentish plover	Saudi Arabia	0.00142283	0.00002328	0.00000001
Two-banded plover	Falklands Islands	0.0271686	0.00211449	0.00003335
Rufous-chested dotterel	Falklands Islands	0.23225174	0.04632724	0.00504751
White-fronted plover	Madagascar	0.03476273	0.00115178	0.00000851
Madagascar plover	Madagascar	0.12828043	0.01495912	0.00070089
Red-capped plover	Australia	0.00204798	0.00002678	0.00000002

Appendix III
Sexual Maturity Relates to Mate Competition and Adult Sex Ratios in Birds

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Manuscript in preparation

This paper reports on original research I helped to conduct during the period of my Higher Degree by Research candidature.

Author contributions

SA: study conception and design, data collection, statistical analysis, manuscript preparation

AL: statistical advice, manuscript improvement

MCC-I: programming advice, manuscript improvement

TS: study conception and design, manuscript improvement

Summary

The age at sexual maturity (i.e., the age at which organisms are physiologically capable of breeding, [1]) has pervasive implications for individual fitness and also for growth and dynamics of populations [2, 3]. Sex differences in age at sexual maturity are common in wild populations [4, 5], although neither the causes nor the consequences of such differences are fully understood [6, 7]. Fisher [8] and Lack [9] proposed that sex difference in maturation is predicted in species where males are under more intense sexual selection, although this hypothesis has not been tested across a wide range of taxa. By using phylogenetic comparative analyses and the most comprehensive dataset to date, we show that sexual selection predicts sex differences in age at sexual maturity: intense sexual selection (as indicated by more polygamous mating and male-biased sexual size dimorphism) is associated with delayed maturation. In addition, we show that adult sex ratios have a knock on impact on age at sexual maturity since delayed maturation in males is associated with overabundance of females in the adult population. Female polygamy, female-biased sexual size dimorphism and male-biased sex ratios predict delayed maturation in females. These results provide the first compelling evidence on how sexual selection and sex ratio variation influence age at sexual maturity in males and females, and open new research avenues to explore how mating systems, life histories and population dynamics interrelate in nature.

Results and Discussion

The Significance of Sex Differences in Age at Sexual Maturity

Age at sexual maturity is closely related to survival and reproduction, and organisms are expected to be sexually mature at the point along their developing trajectories where fitness is maximized [10, 11]. Empirical data on a number of species, including humans, reveal that sex differences in maturation times are common in nature, probably implying that sex specific optima for age at sexual maturity could emerge in a given population as a result of selective factors operating differently on each sex [7, 12, 13]. Nevertheless, sex differences in age at sexual maturity are often overlooked in life history models [4, 5], and neither the causes nor the consequences of sex-biased maturation are fully understood.

Sexual selection is expected to lead to sex differences in maturation times [9, 14-17], and this may occur in two different ways: the sex that experiences a higher level of competition for mates (as occurs for males and females in polygynous and polyandrous mating systems, respectively) could either attain sexual maturity earlier or later than the less competitive sex [17-20]. Both scenarios are plausible for two reasons. By accelerating the attainment of

sexual maturity, individuals (males, for example) would be able to start sexual activities early and secure the best territories and mates [17, 21, 22]. Alternatively, members of the most competitive sex could be forced to delay sexual maturity to fully develop the structures or skills they need to compete successfully for mates [23-25]. Although these ideas were proposed several decades ago to explain sex differences in age at sexual maturity [8, 9], we are not aware of any empirical study testing the relationship between the level of sexual competition that each sex is exposed to, and the age at which males and females are sexually mature. And the few studies on this matter have focused on only one taxon [26, 27], or do not control for phylogenetic relatedness using appropriate statistical tools [21, 28].

Here we examine how sex-specific maturation is influenced by the degree of sexual competition each sex experiences across different mating systems using birds as model organisms. We use the most complete dataset on sex-specific maturation, body size and mating system that currently exists for any organism (201 bird species from 59 families), and a phylogenetic comparative method (phylogenetic generalized least squares, PGLS), to test whether the frequency of male and female polygamy and the level of sexual size dimorphism (SSD), two indicators of the strength of mate competition that each sex experiences in a given mating system [16, 29, 30], predict sex differences in the attainment of sexual maturity.

We also test whether biased adult sex ratio (ASR; the ratio of adult males to adult females in a population) [4, 31] predicts sex-biased maturation in birds. ASR is a demographic property whose variation affects behaviour of animals (e.g., parental care, sex roles, mate competition) [31-33] and humans (e.g., economic decisions, divorce rates) [34, 35], and has important implications for population dynamics and biodiversity conservation [36-39]. Associations between sex-specific maturation and ASR are expected because the social environment may impact on evolutionary trajectories of life histories [40] and breeding strategies [33], for example, by influencing the benefits and costs of initiating reproduction at relatively younger or older ages [41, 42]. Surplus of one sex probably means that members of the rarer sex have more potential partners to mate with [32, 33], and therefore, they may opt for either speeding up sexual maturity and be ready to mate early, or taking more time to be fully prepared to compete and monopolise as many members of the most abundant sex as they can. Investigating how male and female maturation times relate to ASR may provide novel and significant insights into how life histories may respond to variations in the social environment and population numbers.

Polygamy and Sex Differences in Maturation Times

Intense sexual selection is predicted in taxa where males compete for access to multiple mates [43]. However, an increasing number of studies suggests that females may also benefit from multiple mating and polyandry can lead to female-female competition and impose strong sexual selection on female traits [44, 45]. Developmental programmes of both sexes may be subject to the action of sexual selection if by advancing or delaying sexual maturation males and females increase their probabilities of succeeding in mate competition [46, 47]. The most polygamous sex may benefit from early maturation because the earlier an individual matures, the fewer other individuals of the same sex are out there to compete with, and the lower is the number of individuals of the opposite sex that are already paired [29, 48-50]. In marked contrast with this proposition, the most polygamous sex may delay the attainment of sexual maturity because it may need more time to develop and enhance the structures and behaviours required to succeed in intra- and inter-sexual competition for mates [24, 46]. This may include full development of secondary sex characteristics and body size [23, 43, 45].

Our results on birds are consistent with the second scenario: the more polygamous sex attains sexual maturity older than the less polygamous sex across taxa (Figure 1; mean [± SE] of 1000 PGLS models with different phylogenies: slope = 0.069 [<0.001], p <0.001 [<0.001], n = 201 species; see also Figure S1). Furthermore, sex-biased polygamy remains a significant predictor of sex-biased maturity when we control for the effects of life history variables: chick development mode and body mass (Table S1A). These findings have great theoretical significance because they provide the first comparative evidence that strong sexual selection, as indicated by more polygamous mating, can result in delayed maturation, and show that this prediction originally proposed for males several decades ago [8, 9] is equally applicable to females.

Sexual size dimorphism and sex differences in maturation times

Sex differences in reproductive development and maturity may also emerge as a result of the action of sexual selection on male and female body size [46, 51]. Sexual selection is often alluded to explain the occurrence of male-biased SSD (i.e., males are larger than females) in numerous taxa, since enhanced body size often confers advantages to males in mate competition and mate choice [29, 43]. Nowadays, it is well known that sexually selective processes that lead to SSD may act on either sex [23, 45], or they may operate on both sexes at different magnitudes [45, 52]. Therefore, female-biased SSD can be expected in species where females are the sex that competes more intensely for mates [52, 53].

Female-biased SSD may also arise if a smaller adult body size allows males to be more agile when courting, and this increases their breeding success [54, 55].

Differences in developmental trajectories between males and females are often consistent with the direction and magnitude of their dimorphism, so that the larger sex either grows faster or for longer time [46, 56, 57]. Under these two scenarios, the larger sex is expected to delay sexual maturity because somatic growth usually trade-offs against early reproduction [6, 11]. Nonetheless, if the sexes grow under a mix of rates and durations, the fast-growing sex may either stop growing earlier or later than the slow-growing sex, which may translate into a smaller size and earlier maturation, or into a larger size and later maturation, respectively [57-59]. Our results confirm that sex differences in age at sexual maturity relate to SSD, and this association seems to be consistent with sexual selection theory: male-biased SSD and female biased SSD predict delayed maturation in males and females, respectively (Figure 2; PGLS: slope = 0.129 [<0.001], p <0.001 [<0.001], n = 199 species; see also Figure S1). This result is consistent with observations in insects [60], fish [61], turtles [18] and mammals [62] that suggest that the larger sex matures later than the smaller sex. We show that this widely expected association between sex-biased maturation and SSD is robust to phylogenetic relatedness between species, and also to the effects of life-history confounds such as chick development mode and body mass (Table S1B).
We are aware that SSD may emerge as a result of processes other than sexual selection, including fecundity selection for larger-bodied females, natural selection favouring different use of resources by males and females on breeding grounds, or viability selection for small size if large bodies make one sex more vulnerable to starvation, parasitism or predation [7, 56]. In birds, however, SSD seems to be mainly explained by sexual selection operating on males and females [52, 63], and therefore, the relationship between age at sexual maturity and SSD could be assertively attributed to the action of this selective force on developmental trajectories and body size.

Adult Sex Ratios and Age at Sexual Maturity in Males and Females

ASRs exhibit an extraordinary variation across taxa [64], and recent theoretical and empirical evidence suggests that they have a striking impact on mating systems, breeding behaviours and population dynamics [31, 32, 37, 65]. Skewed or variable ASRs are also expected to influence life histories through their impacts on adult survival [39], longevity [66], and breeding strategies [33, 67], but no known study has explored the role that ASR may play in the timing of sexual maturation, in despite a potential association between ASR and maturation times had already been envisioned [24, 68].

Skewed ASRs may translate into limited breeding opportunities or low availability of long-term mates for the supernumerary sex [32, 33], which in response to these conditions could speed up sexual maturation [69], as it may lengthen its reproductive lifespan and increase its breeding opportunities [2, 11]. For the rarer sex, however, skewed environments probably mean multiple mating opportunities, a large amount of variance in mating success, and therefore, strong mating competition [32, 33]. Thus, by delaying sexual maturation, the rarer sex could devote more time and energy to the development of structures and skills required to succeed in mating competition [9, 24], unless accelerated maturation facilitates territory acquisition or maximizes the number of mates [70].

Consistent with expectation, sex difference in age at sexual maturity relates to ASR: delayed maturation in males is associated with overabundance of females in the adult population, and a surplus of males predicts delayed maturation in females (Figure 3; PGLS: slope = -0.081 [<0.001], p <0.001 [<0.001], n = 183 species; see also Figure S1). This relationship remains significant when we control for the effects of chick development mode and body mass (Table S1C). This result is conceptually important because it provides the first comparative evidence that ASR plays a significant role in maturation times, and suggests that sexual maturity can be influenced by the mating opportunities that males and females may exploit in a given population.

Additive Effects of Social Polygamy, SSD and ASR on Sexual Maturity

Sexual selection, as indicated by either sex bias in social polygamy or SSD, and ASR may have additive effects on male and female maturation times. Sex bias in social polygamy and ASR remain significant predictors of sexual maturity when we simultaneously examine the effects of only these two variables (Multipredictor PGLS: sex bias in social polygamy vs. sex bias in sexual maturity: slope = 0.056 [<0.001], p = 0.005 [<0.001]; ASR vs. sex bias in sexual maturity: slope = -0.054 [<0.001], p = 0.005 [<0.001], n = 183 species) and when we control for the effects of additional life-history confounds in multipredictor analyses (chick development mode and body mass; Table S2A). Similarly, SSD and ASR remain significant correlates of sexual maturity in multipredictor analyses that only include these two independent variables (Multipredictor PGLS: SSD vs. sex bias in sexual maturity: slope = 0.097 [<0.001], p = 0.002 [<0.001]; ASR vs. sex bias in sexual maturity: slope = -0.041 [<0.001], p = 0.032 [<0.001], n = 181 species) and that control for the effects of additional life-history confounds (Table S2B).

Although using ASR estimates gathered by different methods often provides consistent results [33, 65, 71], the additive effects of sexual selection and ASR on maturation times are not robust to variation in ASR estimation methods in our sample. Sex-biased polygamy remains a significant correlate of sex-biased maturation, but ASR falls short of significance when we control for the effect of ASR estimation methods in multipredictor analyses (Table S2C). And both SSD and ASR fall short of significance when we include ASR estimation methods as an additional confound in multipredictor PGLS (Table S2D). Nonetheless, lack of information on estimation methods for almost 50% of the ASR estimates considered in our dataset (94 out of 201 species lack this information) reduces our sample size critically, and therefore, this result may be partially due to reduced statistical power.

In conclusion, our results on birds provide the first evidence that sex differences in maturation times may be the result of both the level of sexual competition that each sex experiences in different mating systems [8, 9, 24] and the level of skewedness in ASR, which may signal the number of potential mating opportunities for each sex in a population [31-33]. Sexual selection may favour delayed maturation as it may allow individuals to devote more time and energy to enhanced body size and develop the structures and behaviours that can be key to succeed in mating competition [8, 9, 45]. Nonetheless, since age at sexual maturity is often defined as age of first breeding in the literature, delayed maturation may be partially due to low availability of breeding spots or mates in focal populations [24]. When ASR is biased in a population, the supernumerary sex could increase its breeding opportunities by adopting a different life-history strategy that includes early maturation, analogous to the way menarche is accelerated in female humans in response to limited mating opportunities and low availability of marital partners [69]. In contrast, the rarer sex may experience intense mating competition for access to a greater number of mates [32], and therefore, it may require longer to mature. Further research is probably needed in other taxa that exhibit marked variation in maturation times, sex ratios, body size and sexual selection (e.g., mammals [20, 23]) to better understand the generality of these patterns, as well as experimental manipulations examining causal relationships between ASR, sexual selection and life histories. Investigating underlying causes of variation in the age at sexual maturity is important because this trait may have important implications for demography and population persistence [3, 4].

Experimental Procedures

We conducted an extensive literature search to assemble published data on age at sexual maturity, body size and sexual competition for males and females, as well as data on ASR

currently available for wild bird populations. Age at sexual maturity (in months) was often defined as age of first breeding, although other criteria were also used, including the level of gonadal maturity and the age when secondary sex characteristics are fully developed. Sex bias in age at sexual maturity was estimated as log(male age at maturity/female age at maturity). Sexual competition was expressed as the incidence of social polygamy, and it was estimated for both sexes separately using a five-point scoring system (score 0, <0.1% of individuals are polygamous; score 4, >20% of individuals are polygamous). We estimated sex bias in the level of polygamy as the difference between the male and the female polygamy scores. Body size, the mean body mass in grams of adult males and adult females, was used to estimate SSD as log(adult male mass/adult female mass). ASR was estimated by different methods, including censuses and captures of breeding and non-breeding adults, counts of birds dying from natural causes and demographic modelling. Different data availability for different variables prevented us from using the same sample sizes in all analyses.

To analyze interspecific data, we used Phylogenetic Generalized Least Squares with maximum likelihood estimates of Pagel's λ values [72, 73] as implemented in R packages "ape" and "caper" [74]. To account for phylogenetic relationships, we used one thousand trees randomly selected from the latest and most comprehensive avian phylogeny [75]. Full details of our methods are provided in the Supplemental Experimental Procedures; the complete dataset, together with their sources, is provided in Table S3.

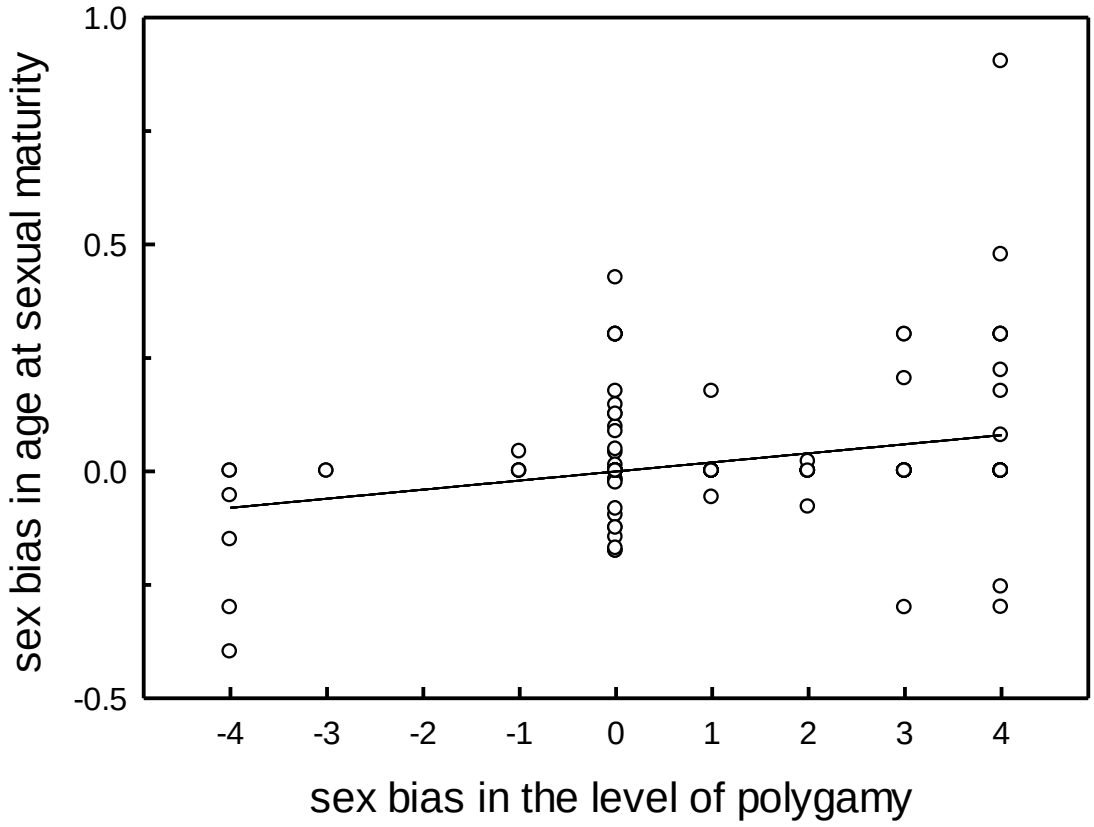

Figure 1. Sex bias in age at sexual maturity (log(male age at maturity/female age at maturity)) is associated with sex differences in the level of polygamy. Polygamy incidence was scored for each sex on a five-point scale, from 0 to 4, and sex bias in the level of polygamy was estimated as male – female polygamy score. Regression line is the mean slope of phylogenetic regressions (PGLS) fitted with 1000 different phylogenetic hypotheses (n = 201 species; see text for statistics and Figure S1 for supplemental results).

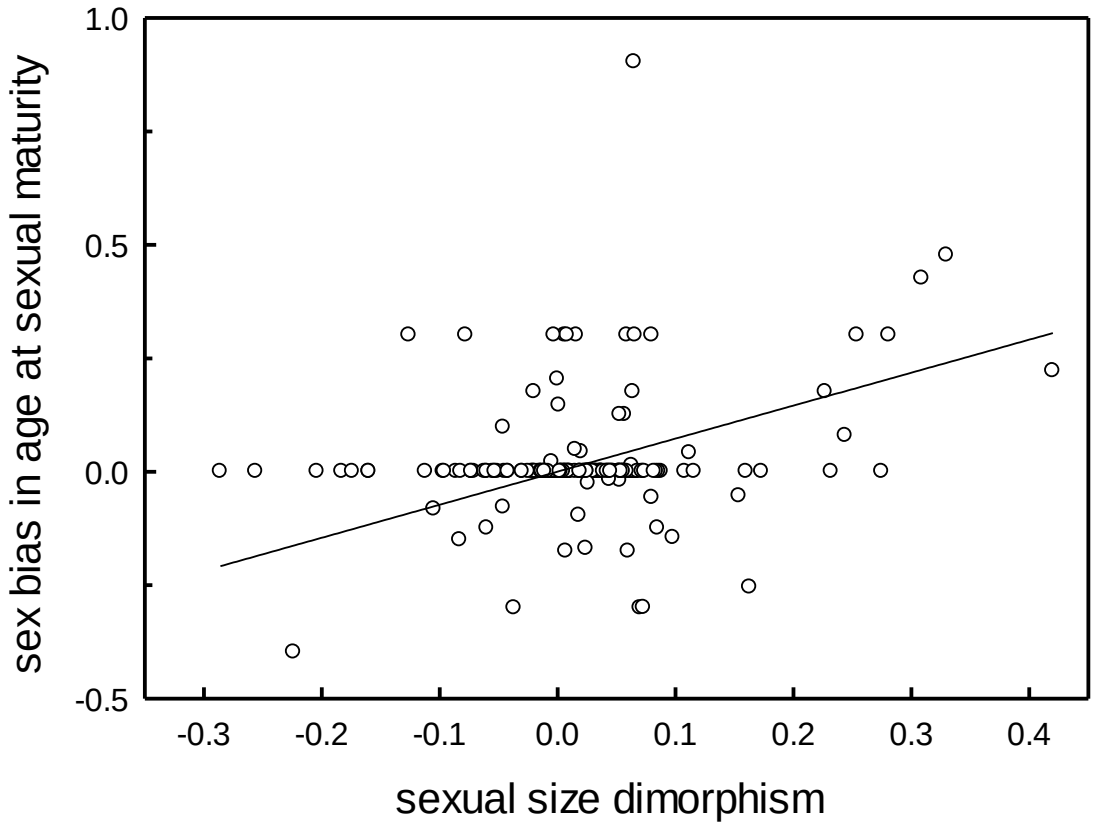

Figure 2. Sex bias in age at sexual maturity in relation to sexual size dimorphism (log(adult male mass/adult female mass)) across 199 species of birds. Regression line is the mean slope of phylogenetic regression models (PGLS) using 1000 different phylogenetic hypotheses (see text for statistics and Figure S1 for supplemental results).

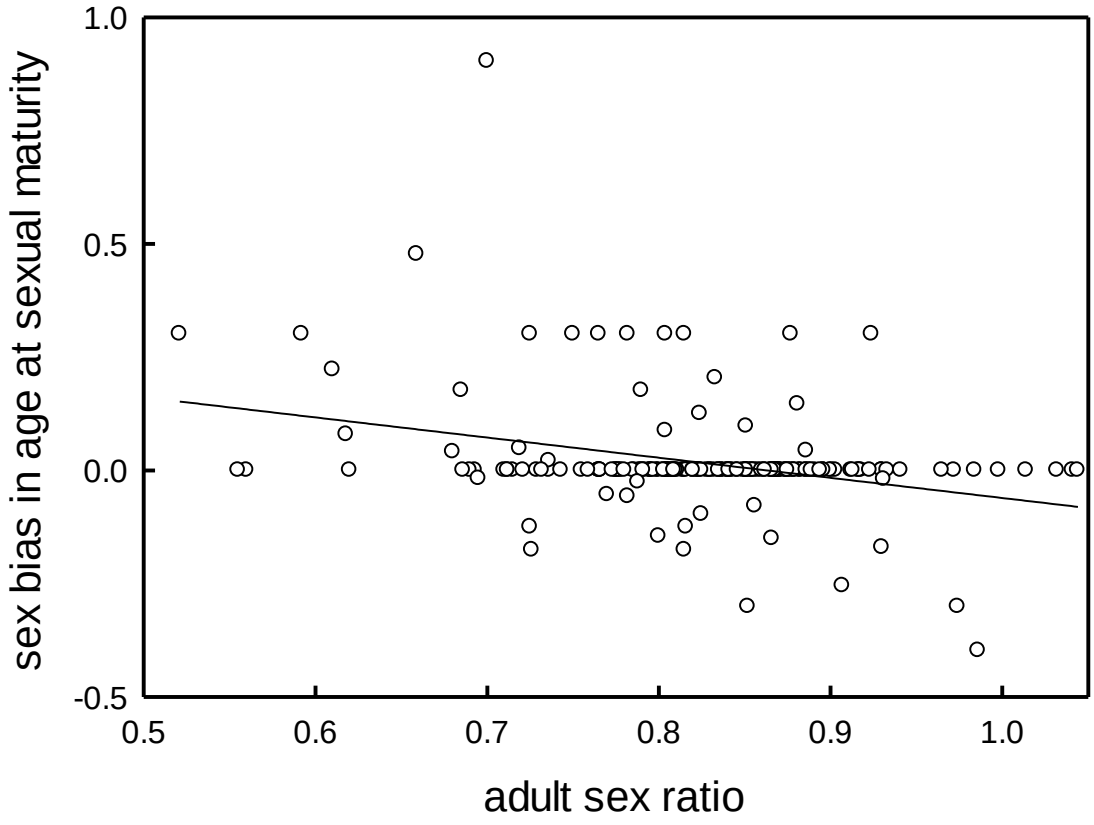

Figure 3. Adult sex ratio (ASR) predicts sex differences in age at sexual maturity. ASR was estimated as the number of adult males/(number of adult males + number of adult females), and its arcsine-square-root-transformed values are shown. Plot shows species values (n = 183), and the regression line was fitted by PGLS models using 1000 different phylogenies (see text for statistics and Figure S1 for supplemental results).

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Supplemental Information

Supplemental Data

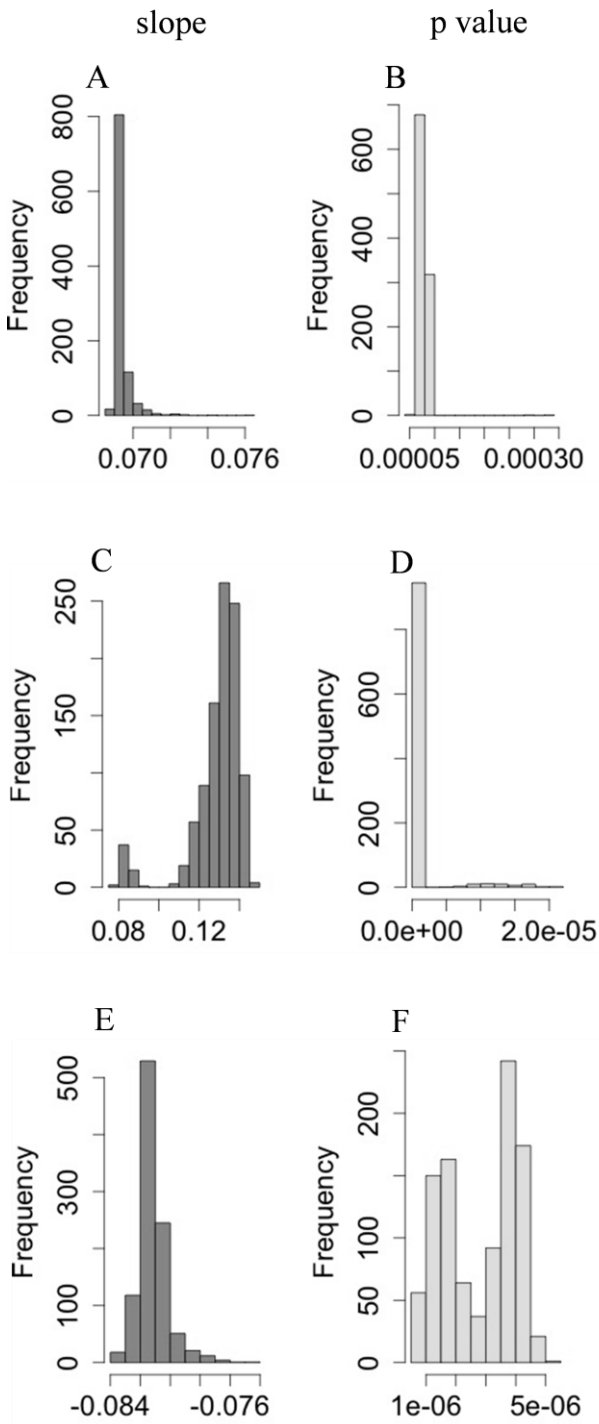

Figure S1. **Related to Figures 1 – 3**

Distribution of slopes (red histograms) and p values (green histograms) of bivariate PGLS models fitted with 1000 different phylogenetic trees. A – B: sex-biased maturity vs. sex-biased polygamy; C – D: sex-biased maturity vs. sexual size dimorphism (SSD); E – F: sex-biased maturity vs. adult sex ratio (ASR).

Table S1. Supplemental analyses testing the robustness of significant bivariate correlations between sex-biased maturation and three independent variables: sex-biased polygamy, SSD and ASR to the effects of two life-history confounds.

Slope and p values are shown as the mean [± SE] of 1000 Phylogenetic Generalized Least Squares models (PGLS) with different phylogenies, n = number of species. Detailed description of variables and analyses are provided in Supplemental Experimental Procedures.

Model / predictor variables	slope	p	n
(A) *Sex-biased polygamy + life-history confounds*			
Sex-biased polygamy	**0.070 [<0.001]**	**<0.001 [<0.001]**	199
Chick development mode	**-0.049 [<0.001]**	**0.034 [0.001]**	
Average body mass	0.040 [<0.001]	0.081 [<0.001]	
(B) *SSD + life-history confounds*			
SSD	**0.122 [<0.001]**	**<0.001 [<0.001]**	199
Chick development mode	-0.024 [<0.001]	0.671 [0.005]	
Average body mass	0.031 [<0.001]	0.469 [0.004]	
(C) *ASR + life-history confounds*			
ASR	**-0.078 [<0.001]**	**<0.001 [<0.001]**	181
Chick development mode	-0.027 [<0.001]	0.456 [0.006]	
Average body mass	0.017 [<0.001]	0.503 [0.006]	

Table S2. Supplemental analyses testing the robustness of significant additive effects of sexual selection (as indicated by either polygamy bias or SSD) and ASR on male and female maturation to the effects of life-history confounds (A, B) and variation in ASR estimation methods (C, D).

We report slope and p values as the mean [± SE] of 1000 PGLS with different phylogenies, n = number of species. Detailed description of variables and analyses are provided in Supplemental Experimental Procedures.

Model / predictor variables	slope	p	n
(A) *Sex-biased polygamy + ASR + life-history confounds*			
Sex-biased polygamy	**0.055 [<0.001]**	**0.007 [<0.001]**	181
ASR	**-0.054 [<0.001]**	**0.007 [<0.001]**	
Chick development mode	-0.030 [<0.001]	0.264 [0.004]	
Average body mass	0.011 [<0.001]	0.746 [0.007]	
(B) *SSD + ASR + life-history confounds*			
SSD	**0.095 [<0.001]**	**0.002 [<0.001]**	181
ASR	**-0.042 [<0.001]**	**0.033 [<0.001]**	
Chick development mode	-0.007 [<0.001]	0.871 [0.006]	
Average body mass	0.002 [<0.001]	0.819 [0.005]	
(C) *Sex-biased polygamy + ASR + ASR estimation methods*			
Sex-biased polygamy	**0.040 [<0.001]**	**0.045 [<0.001]**	109
ASR	-0.035 [<0.001]	0.093 [<0.001]	
ASR estimation methods	-0.018 [<0.001]	0.308 [<0.001]	
(D) *SSD + ASR + ASR estimation methods*			
SSD	0.044 [<0.001]	0.054 [<0.001]	107
ASR	-0.037 [<0.001]	0.070 [0.001]	
ASR estimation methods	-0.027 [<0.001]	0.158 [0.003]	

Table S3. Related top Experimental Procedures

The full data set and data sources are provided in a separate Excel table.

Supplemental Experimental Procedures

Sex bias in age at sexual maturity

We systematically looked for data on age at sexual maturity for males and females in reference works (e.g., The Birds of the Western Palaearctic and Birds of North America) and the primary literature through the Web of Knowledge and Google Scholar using Latin and English names of specific taxa in combination with “age at maturity”, “age at first reproduction” and “recruiting age”. First, we focused our search on 187 species for which Székely, et al. [71] assembled data on male and female body size, sexual competition and ASR in order to maximize the completedness of the dataset with regard to the working hypotheses. Data on sex-specific age at sexual maturity are uncommon, and the number of species showing sex differences in the attainment of maturity was particularly limited in this initial dataset. Therefore, in order to guarantee an adequate amount of variance in the data, we extended the sampling to all other bird species that may exhibit sex differences in the attainment of maturity according to AnAge, a curated online database of vertebrate life histories [76]. We accessed all original sources referenced in AnAge and only kept those data on sex-specific age at maturity that came from peer reviewed hadbooks and scientific publications. We obtained data on sex-specific age at sexual maturity (months) for a total of 201 species. The complete dataset, together with their sources, is provided in the electronic supplementary material, table S1.

The age at sexual maturity was often defined as age of first breeding, although other criteria were also used, including the level of gonadal maturity and the age when secondary sex characteristics are fully developed. We aimed to use the best data available, and we are not aware of any systematic bias that would undermine our working hypotheses. When several estimates were available for a given species (e.g. from different subspecies or from different studies), we used the ones based on larger sample sizes and estimated their mean value. We express sex bias in age at sexual maturity as log(male age at maturity/female age at maturity).

Sexual size dimorphism and sex bias in the level of polygamy

We augmented the data on body size and sexual competition for both sexes originally assembled by Székely, et al. [71]. Body size was the mean body mass in grams of adult males and adult females, and SSD was computed as log(adult male mass/adult female

mass). Our dataset includes SSD estimates for 199 species that also have data on sex-specific maturation.

We scored the frequency of polygamy for each sex on a five-point scale (0 – 4) where '0' denotes no or very rare polygamy (<0.1% of individuals), '1' rare polygamy (0.1 – 1%), '2' uncommon polygamy (1 – 5%), '3' moderate polygamy (5 – 20%) and '4' common polygamy (>20%) (see details in [33, 77]). When the frequency of polygamy was not provided in the original source, we scored the frequency of polygamy based on verbal descriptions of the mating behaviour and pair bonds available for focal species. Scoring was essential to include as many species as possible in the analyses, and it was statistically consistent between observers (intraclass correlation, $r_{ICC} = 0.914$, $F = 22.2$, $p < 0.001$, $n = 28$ species). When different indices of polygamy were available for a focal species, we used their average value. We estimated sex bias in the level of polygamy as the difference between the male and the female polygamy scores, as it has been estimated in previous analyses [65, 71]. Sex bias in the level of polygamy was estimated for all 201 species for which we obtained data on sex-specific maturation.

Adult sex ratio (ASR)

The dataset originally assembled by Székely, et al. [71] includes 187 species and reflects information on ASR currently available for birds. We obtained data on sex-specific maturation for 176 species listed in this initial dataset. In addition, we looked for data on ASR for those 14 species we added to this initial dataset for which information on sex-specific maturation was available. Our final sample includes ASR estimates for 183 species that also have data on sex-specific maturation. We followed the same criteria that Székely, et al. [71] used to collect data on ASR. When several ASR estimates were available for a species (e.g., from different years or different populations), we calculated their average value.

We used ASR estimates obtained by different methods, including censuses of individually marked breeding adults, captures of breeding and non-breeding birds, counts of birds dying from natural causes (e.g., storms), counts of museum specimens and demographic analyses (such as [78] and [79]). These ASR estimates obtained from different populations and gathered by different methods are expected to provide consistent results [33, 65, 71]. We excluded, however, ASR estimates based on counts of hunted birds, and preferred estimates that were likely least influenced by habitat loss and other anthropogenic impacts. We used ASR estimates as provided in the original sources, although for 15 species we

computed ASR from tables or figures offered in these sources to report the number of adult males and females in a given population. Consistently with previous studies, ASR was estimated as the number of adult males/(number of adult males + number of adult females), and it was arcsine-square-root-transformed before analysis [33, 71].

Statistical analyses and confounding variables

First, we tested whether sex bias in age at sexual maturity can be predicted by either sex bias in the level of polygamy, SSD or ASR using separate bivariate Phylogenetic Generalized Least Squares (PGLS) with maximum likelihood estimates of Pagel's λ values [72, 73]. To represent the phylogenetic relationships between species, we used the latest and most comprehensive avian phylogeny that includes all 201 species in our dataset. To test the sensitivity of results to phylogenetic uncertainty, we used a sample of 1000 phylogenetic tress, which we extracted randomly from the 10 000 alternative phylogenetic hypotheses available at http://birdtree.org, using the sample tool offered on this website. One thousand trees is suggested as a robust sample to reduce potential errors associated with phylogenetic uncertainty [80]. All phylogenetic trees were fully resolved (i.e., did not contain polytomies) and included branch lengths (see details in [75]). We repeated each PGLS model with each of the 1000 trees and calculated the mean slope and mean two-tailed significance levels of the phylogenetic regressions, which we provide together with their standard errors (SE). We also report the distribution of slopes and p-values for all bivariate phylogenetic regressions.

In a second step, we used a set of three multi-predictor PGLS to test whether sex-biased polygamy, SSD and ASR (separately) remain significant correlates of sex-biased maturation after controlling for the potential confounding effects of two life history traits: chick development mode and adult body mass. Thus, each multi-predictor model included these two life-history confounds along with one of the three predictors of interest, either sex-biased polygamy, SSD or ASR. Data on offspring development mode were collected from published literature, categorised as (0) altricial, (2) semi-altricial or semi-precocial, or (3) precocial, and included as a 3-level factor in PGLS. Adult body mass (in g) was computed as the mean male and female masses and log 10-transformed before the analyses.

To test whether the intensity of sexual competition, as indicated by either sex bias in social polygamy or SSD, and ASR have additive effects on male and female maturation times, we used two sets of multi-predictor PGLS. First, we constructed a model containing sex-biased polygamy and ASR as predictors of sex-biased maturation. Then, we constructed a separate model including SSD and ASR as predictors of sex-biased maturation. In a further step, we

repeated these two sets of multi-predictor PGLS and included both chick development mode and adult body mass as additional predictors of sexual maturation. This allowed us to test whether additive effects of sexual competition and ASR on sexual maturation remain significant after controlling for potential confounding effects of life-history traits.

Finally, we used two additional sets of multi-predictor PGLS to test whether significant additive effects of sexual competition and ASR on sexual maturation are robust to heterogeneity in ASR estimation methods. ASR estimation methods were categorised as (1) counts of non-breeding birds, (2) counts of breeding birds, (3) counts of breeding and non-breeding birds, (4) counts of birds dying from natural causes/counts of trapped birds, (5) estimates based on demographic modelling, and (6) estimates based on counts of museum specimens. We included ASR estimation methods as a 6-level factor in PGLS along with either sex-biased polygamy or SSD, and ASR.

To produce comparable effect sizes, we calculated standard PGLS parameter estimates (slopes) by scaling all the predictors according to the method proposed by Gelman [12]. Different data availability for different variables prevented us from using the same sample sizes in all PGLS. All reported significance values are two-tailed since we performed PGLS that did not assume directionality in the relationships between variables. We dropped non-significant interactions before calculating mean slopes and significance levels of main terms included in multipredictor PGLS. We calculated the variance inflation factor (VIF) for all models; this VIF was < 1.5 for all models, suggesting that multi-collinearity may not inflate results. All analyses were carried out in R using packages ‘ape’ and ‘caper’ [74, version 3.0.1].

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